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An Update of the 1975 Report

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REVIEW AND EVALUATION OF AVAILABLE TECHNIQUES FOR DETERMINING PERSISTENCE AND ROUTES OF DEGRADATION OF CHEMICAL SUBSTANCES IN THE ENVIRONMENT: AN UPDATE OF THE 1975 REPORT

by

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This report updates a 1975 study (EPA 68-01-2210 - Final Report NTIS PB 243-825) that reviewed and evaluated the present state of techniques which have been used to determine the environmental persistence and pathways of degradation of chemicals released into the environment. Both chemical and biological routes of degradation in the environment were considered. New developments since the 1975 report included validation and round-robin testing of biodegradation tests and the development of screening tests that did not require specific chemical analysis. Considerable progress was also made in understanding the mechanism and rates of some non-biological degradation processes.

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I. INTRODUCTION

A. General

This report is an update of a report prepared by the Syracuse Research Corporation in 1975 (Howard et al., 1975) on techniques used to determine the persistence and degradation of chemicals in the environment. For the most part, this report covers new material, although in some cases material from the old report has been repeated. The first two chapters are introductory in nature and with some slight revisions were abstracted basically from the earlier report. The other three chapters on Aqueous Biodegradation, Soil Degradation, and Photochemical and Chemical Alterations cover mostly new material. One exception to this is the section on Atmospheric Degradation (V-A).

A major factor for assessing the potential hazard of these environmental contaminants is the consideration of the chemicals' environmental persistence and degradation products. For example, it is quite possible that "highly toxic, readily biodegradable substances may pose much less of an environmental problem than a relatively harmless persistent chemical that may well damage a critical wild species" (Goodman, 1973). This report reviews and evaluates techniques that have been used to determine the environmental persistence and degradation pathways of various chemicals.

The importance of knowing the alterations and ultimate fate of a chemical in the environment can vary depending upon the chemical. Understanding the environmental alterations of metals and organometallic compounds is required because the toxicity and mobility of the material may be affected greatly by various changes in chemical form. In addition, the kinetics of the alteration reactions in the environment may be extremely important since some of the degradation routes (e.g. methylation-demethylation) are reversible (Wood, 1974).

With organic compounds, environmental reactions are usually irreversible, and furthermore, the organic compounds can potentially degrade to water and carbon dioxide. For organic compounds, the term "persistence" has much more relevance. The kinetics of the reactions have received considerably more attention than in our earlier report, although some of the approaches proposed involve considerable difficulties. This is undoubtedly due to the fact that modelling the dynamic environment in the laboratory is most difficult in that the environment consists of numerous microenvironments that can vary considerably in temperature, pH, microbial population, etc. Thus, quantitative data developed under one set of laboratory conditions may be somewhat misleading for another set of environmental conditions. As a result qualitative indications of environmental degradation continue to be used for screening purposes because they may be relied upon in many different microenvironments.

Some definition of nomenclature is necessary. Such terms as environmental persistence, alteration, biodegradability and degradability are lacking in precision and often are dependent upon the perspective of the author. Soil scientists frequently refer to pesticides that are not retained in the soil for long periods of time as non-persistent, although, in many cases, the loss may be due to physical removal processes, such as vaporization and leaching (resulting in dilution but not destruction). Biodegradability is frequently considered by detergent scientists as synonymous with treatability or removability in a sewage treatment plant. The interpretation of these terms as they are used in this report is reviewed briefly.

The terms persistent and degradable are applied in this report only to the total environment rather than to any individual medium (air, soil, water). Therefore, any transport processes of a chemical are irrelevant to a determination of persistence or degradability unless it may change the possible reactions that may take place (e.g., vaporization into the atmosphere almost eliminates the possiblity of biodegradation). Degradation means "to reduce the complexity of a chemical compound by splitting off one or more groups or larger component parts" (WPCF, 1967). Biodegradation means that the destruction process is accomplished by the action of living organisms (e.g., see Swisher, 1970). Environmental alteration means that the compound has undergone a chemical change that is not necessarily a reduction in the complexity of the molecule.

Biodegradation has been divided further into the following three categories (WPCF, 1967):

> "1. Primary Biodegradation - Biodegradation to the minimum extent necessary to change the identity of the compound.
>
> 2. Ultimate Biodegradation - Biodegradation to (a) water, (b) carbon dioxide, and (c) inorganic compounds (if elements other than C, H and O are present).
>
> 3. Acceptable Biodegradation - Biodegradation to the minimum extent necessary to remove some undesirable property of the compound such as foaminess or toxicity."

Primary biodegradation is basically the loss of the parent compound, although this is somewhat dependent upon the analytical methods used. Ultimate degradation is the complete mineralization of the organic chemical. OECD (1981) has referred to primary biodegradation as that measured by "specific analysis" of the test chemical and ultimate biodegradation or mineralization as that measured by "non-specific analysis" (e.g., CO_2 evolution, methane, or dissolved organic carbon). The third category falls between the two extremes but results in perhaps the haziest definition. In another approach, Dagley (1972) has suggested that a man-made compound is biodegradable if it is attacked by the enzymic apparatus acquired by microbes during the course of evolution. Painter (1973) has noted that two general criteria have been used to assess the biodegradability: (1) the rate and extent to which natural or enriched mixed cultures degrade the compound, and (2) the proportion of tested species, strains, or isolates that are able to use the compound as a sole source of carbon and energy. Prochazka and Payne (1965) feel that the ability to isolate microorganisms that use the test compound as a sole source of energy and carbon is the best criteria for biodegradability.

These definitions are obviously only of a qualitative nature. As mentioned earlier, this is due to the numerous chemical compounds to which the definitions are applicable and the lack of precision inherent in biological systems. This non-quantitative nature is in contrast to the half-life of an isotope, for example, where the rate is independent of environmental events (Dagley, 1972). Thus, assessment of a chemical's persistence or degradation rate in the environment requires that the compound be evaluated in some type of test method or technique, and the results are only meaningful when stated with the conditions of the technique. The components essential to such techniques are the test chemical, the analytical method, and the environmental component. A good test method should meet the following criteria: (1) be as simple as possible, (2) be as economical as possible, (3) be reproducible, and (4) report results that can be correlated to field conditions (see SDA, 1965). These components of various techniques are reviewed and evaluated in detail in the following sections.

Well over 44,000 synthetic organic chemicals are produced in commercial quantities (U.S. EPA, 1979) and annually total approximately 140 billion pounds in the United States (U.S. Tariff Commission). Iliff (1972) has suggested that, world-wide, up to 40 billion pounds of manufactured organic chemicals enter the enviroment annually. Included in this estimate are organic compounds used as pesticides (over 1 billion pounds, U.S. Tariff Commission), which are intentionally distributed in the environment, detergents (Swisher, 1970; Anon., 1963), solvents (Levy, 1973; Laity _et al_., 1973), plastics (39 billion pounds in

1978), chemical intermediates (20 billion pounds in 1978), plasticizers (2 billion pounds in 1978) and other compounds, that may be inadvertently or accidentally released during manufacture, transport, use, and/or disposal. Compounds, such as polychlorinated biphenyls (PCB's), phthalate esters, hexachlorobenzene, and other organic chemicals (e.g., Miller, 1973; Kleopfer and Fairless, 1972; A.D. Little, Inc., 1970), are typical of the latter category. In addition, it has been estimated that more than 2 billion pounds of oil are lost at sea annually (Friede *et al*., 1972) and about 9.5 billion pounds of total plastic wastes accumulate each year (Potts *et al*., 1972).

These different categories of organic chemicals enter and reside in different parts of the environment. For example, detergents usually pass through a biological treatment plant before entering streams, rivers, lakes, etc. Pesticides are normally sprayed on crops or, if they are aquatic herbicides, they may directly enter aqueous systems. Those solvents that are fairly volatile vaporize into the atmosphere. From the initial entrance point, all of the above materials may be biologically or chemically altered or physically transported from one point to another. Understanding the mobility and physical transport mechanisms of a chemical is important in terms of determining the potential effect of the environmental contaminant; however, as long as the pollutant remains in existence, it can enter the food chain or affect man directly (Alexander, 1967). On the other hand, if the organic compound is completely mineralized to inorganic material (CO_2,H_2O), its potential for being a pollutant is removed. The possible biological and chemical degradation processes that may take place in the environment are reviewed in Section II. In addition, biodegradation of chemicals under biological treatment conditions is considered since a compound's fate in these systems can be the determining factor in whether the compound becomes an environmental contaminant or degraded to innocuous materials.

B. Methods and Approach

1. Literature Search

Papers, books and review articles relevant to this review were gathered by a variety of approaches. Extensive use was made of such abstracts as the Air Pollution Abstracts, Chemical Abstracts, and Pollution Abstracts, in an effort to identify relevant articles published since 1975. Because these abstracting services all have some amount of delay between the time when the article is published and the time when it appears in the abstract, a search of recent, highly relevant journals using Current Contents was also undertaken. In addition, a computer search was performed for the reports published by the National Technical Information Service (NTIS) and for the current investigator files of the Smithsonian Science Information Exchange (SSIE).

The abstracts, obtained in this way were screened for relevant articles, and ~~the~~ papers deemed pertinent were collected and examined. The bibliography of highly relevant articles was examined to provide a double check on the abstract search. In some instances, a citation search of extremely pertinent articles was conducted using Science Citation Index.

Individual researchers, who were either professional acquaintances of the authors of this report or were identified by the SSIE computer search, were contacted personally and requested to supply any articles that they had published recently or had in press. In addition, in a number of cases, personal visits were made to some researchers' laboratories in order to discuss experimental techniques used. Furthermore, a number of experts in the field have been asked to review drafts of the final report.

The cost of the various techniques have been reviewed in a limited number of articles (OECD, 1980; U.S. EPA, 1978) since 1975 and, therefore, these prices, as well as updates of our estimates, are presented. Information used to calculate our estimates included (1) time requirements cited in papers, obtained from researchers, or estimated by the authors of this report, (2) the complexity of the procedure and necessary technical training of the personnel, and (3) capital investments in equipment. The cost may fluctuate considerably depending upon the chemical being tested and the laboratory doing the testing and, therefore, the projected costs have only an order of magnitude reliability.

2. Scope and Organization of the Report

This report reviews techniques that have been used to study the persistence and breakdown of chemicals in the water, soil, and air medium of the biosphere. With the exception of the model ecosystem technology, techniques used to study metabolism of chemicals in higher trophic levels (e.g., plants, fish, mammals) have been excluded. It was felt that these processes, although important to considerations of bioaccumulation and toxicity, are less relevant to environmental degradation and persistence. A full discussion of this rationale is presented in Section II A.

This report is organized by category. For instance, biodegradation is divided by different media (e.g., soil, water). In one instance, the type of degradation (chemical and photochemical) is the distinguishing factor. For the most part, these categories were chosen as a matter of convenience in reviewing the available information. Most sections are divided into (1) a review of the techniques, (2) analytical techniques, (3) evaluation of the techniques, and (4) a cost analysis.

The quality and quantity of the information has varied considerably depending upon the chemical and the medium. By far, the most intensively studied group of chemicals in their appropriate medium have been the pesticides and commercial organics in soil and water, the detergents in sewage and water, and the hydrocarbons in the atmosphere. The only change from our 1975 report has been the increase in the number of commercial synthetic organics that have been studied. The approaches and techniques used with these materials have been reviewed for their applicability to other chemical groups of potential environmental contaminants.

Most of the techniques reviewed are applicable to a determination of degradability or persistence. Techniques for studying the routes of degradation have not been reviewed in a great amount of detail because they are very similar to procedures used to study metabolic pathways of natural substrates.

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II. TYPES OF DEGRADATION IN THE ENVIRONMENT

Chemicals placed in the environment are subjected to chemical alterations and physical processes by sunlight, water, soil, inorganic and organic material, and biological agents and by the combined weathering action of rain, wind, temperature and humidity (Van Middelem, 1966). The physical processes, such as adsorption on colloidal substances, volatilization, bioaccumulation or leaching, have a tendency to distribute and dilute or concentrate but not destroy the contaminants. On the other hand, chemical and biological reactions in the environment result in alterations and frequently degradations of the material to innocuous substances. The alteration and degradation processes can be divided into three general categories: (1) biodegradation - affected by living organisms; (2) photochemical degradation - nonmetabolic degradation requiring light energy; and (3) degradation affected by chemical agents (chemical degradation) - nonmetabolic degradation which does not require sunlight. Alexander (1967) has suggested that of these three, biological degradation of organic compounds is the most desirable because it results generally in end-products that have been completely mineralized to inorganic compounds. In contrast, photochemical and other nonmetabolic processes usually result in only slight modifications in the parent compound; however, all of these processes are important from the standpoint of the environmental fate of the chemicals, and will be discussed in detail in the following sections.

A. Biological Degradation

In order to insure growth, maintenance and development, living systems must obtain carbon, other essential elements, and energy from their environment. For non-photosynthetic organisms, this process involves the absorption of various naturally occurring organic compounds, the subsequent metabolism of these compounds to yield energy and essential elements that can be incorporated into the structure of the organism, and the elimination of metabolic products. Although biological transformations of organic chemicals generally occur via relatively specific processes, there is sufficient latitude or non-specificity in many organsisms to allow them to perform a significant role in the degradation and/or removal of various synthetic chemicals that are introduced into the environment. The factors governing this ability to degrade a natural or synthetic compound are: (a) the compound must be able to reach the organism or the enzyme site; (b) the compound must not be lethal; (c) the enzymes necessary to alter the chemical must be present or able to be induced; and (d) the environmental conditions must permit the operation of the enzymes (Alexander, 1966). Thus, if absorbed by a given organism, a synthetic chemical may be (a) degraded to serve as a source of carbon and/or energy; (b) degraded by cometabolic processes (see Horvath, 1972); (c) slightly modified and then stored or excreted; and (d) stored or excreted without chemical alteration. Frequently there is a lag between the exposure of the chemical to the biological agent and the beginning of degradation or transformation. In the case of microorganisms, the lag is attributed to the need for "acclimation." This term will be frequently referred to in this report and, therefore, needs to be defined. Acclimation is a broad term, usually referring to a variety of processes (such as enzyme induction, selection of a species of microorganism, etc.) that take place during the lag period.

Although biological degradation conceivably might be accomplished by any living organism, available information indicates that by far the most significant biological systems involved in ultimate biodegradation - degradation to CO_2, H_2O, and other inorganic compounds - are the bacteria and fungi. This conclusion is based on (1) what is known about the metabolic pathways of bacteria, fungi and higher organisms; (2) the projected metabolic activity of various organisms; and (3) the availability of synthetic organics to the bacteria and fungi. The following paragraphs are devoted to an elaboration of the factors listed above.

1. Metabolic Pathways

Various forms of life including wildlife, fish, various marine species, domestic animals, and even man have been shown to contain some residues of environmental chemicals (Smith and Isom, 1967; Davis and Hughes, 1965; Rawls, 1966; Durham, 1969; Edwards, 1973; Holden, 1970). Whether the eventual loss of chemicals from these organisms is due to metabolism or excretion is unclear. Loeffler and Van Overbeek (1971) have stated that "organisms with an effective excretory mechanism [which perhaps includes most animals] will, as a rule, not degrade the compound that does not fit into the normal sequence of metabolic events as completely as do those organisms in which this means of disposing of a compound is absent." This is based on the fact that a compound that is sufficiently water soluble (allow excretion) requires less energy for excretion than does the large number of reactions required to degrade a compound into small molecular fragments or even gaseous products (Loeffler and VanOverbeek, 1971). On the other hand, water insoluble compounds (e.g., chlorinated hydrocarbons and insecticides) tend to accumulate, and storage of residues often occurs in the adipose tissue of the organism (Edward, 1973; Kenaga, 1972).

Higher plants usually do not have an efficient excretion apparatus. The metabolic tendency in such cases is to convert the chemical into some neutral, water-soluble form in which it can be stored in cell vacuoles (Crosby, 1973). Examples of these transformations prior to storage include (1) conversion of naphthalenacetic acid to its glucose ester, followed by the formation of the more stable amino acid conjugate (Veen, 1966); (2) conversion of the insecticide carbaryl into persistent glycosylated metabolites (Kuhr and Casida, 1967); and (3) binding of certain pesticides to plant proteins (Brian, 1960) or incorporation into structural lignins, pectic substances, etc. (Chin et al., 1964; Meagher, 1966).

In contrast to the metabolic processes of higher organisms, the catabolic versatility of bacteria and fungi suggests that these organisms are likely to play a major role in the ultimate degradation of synthetic chemicals that enter the environment. Alexander (1973) has stated that all evidence points to the fact that microorganisms are responsible for converting to inorganic products many complex natural and synthetic organic molecules that cannot be significantly altered by higher life forms. Enzymatically, the degradative activity of bacteria and fungi may be based on their ability to catalyze the initial steps in degradation resulting in metabolites that can enter existing metabolic pathways (Dagley, 1972a). Furthermore, microbial communities in the environment seem to vary more in the number of different microbial groups present. This high species diversity in microbial communities results in a multitude of possible biochemical pathways which may provide a major advantage to microorganisms over other organisms in catalyzing degradation of the many structurally different environmental chemicals.

Invertebrate forms including the protozoans and lower metazoans have not been studied as intensively as the vertebrates, higher plants, bacteria, and fungi. These invertebrates have not been directly implicated as yet in the ultimated degradation of synthetic chemicals although they are associated with the dispersion of such compounds (Wright, 1971) and thus make the chemicals more accessible to the bacteria and fungi (Alexander, 1966).

A great number of studies can be cited in which a compound has been found to be degraded by the action of microorganisms but not by higher organisms. Funderburk (1969) reported no metabolism of paraquat in higher plants. Numerous microorganisms have been found, however, to use paraquat as a sole source of carbon or nitrogen (Baldwin et al., 1966; Anderson and Drew, 1972). DDT, a pesticide well known to be persistent in organisms of higher trophic levels (Edwards, 1973), has been shown to undergo extensive degradation by the combined action of two bacteria, as shown by in vitro studies of Pfaender and Alexander (1972). Another good example is the herbicide endothal. Sikka et al. (1974) have shown that bluegills removed less than 1% of the total amount of herbicide; the removed amount was present in the fish in unchanged form. Shake culture and aquarium studies (Sikka and Saxena, 1973; Sikka and Rice, 1973), however, have shown that endothal is rapidly degraded by microorganisms. When mixed-function oxygenases from liver microsomes (from animals) attack naphthalene, an epoxide is formed which may isomerize to give naphthol, or undergo enzymic hydrolysis to the trans-dihydrodiol; the product formed is usually excreted as conjugates (Gibson, 1972; Dagley, 1972b). By contrast, microorganisms oxidize naphthalene and break down the rings to provide a source of carbon and energy for growth (Gibson, 1972; Dagley, 1972b).

Degradation of several natural materials by microorganisms can further substantiate the unique role of microorganisms in governing environmental persistence of chemicals. Pristane, an isoprenoid alkane that is formed by crustaceans of the genus Calanus, has been found to accumulate unchanged in the locations where it is not susceptible to microbial attack (McKenna and Kallio, 1971; Dagley, 1972b). Under favorable conditions, however, pristane is readily metabolized by a variety of microorganisms (McKenna and Kallio, 1971; Dagley, 1972b). Another natural product, atropine, that is probably even more toxic than many pesticides, has been found to serve as a source of carbon for certain microorganisms (Niemer et al., 1959; Niemer and Bucherer, 1961). In other living forms, these compounds evoke mechanisms of detoxification and elimination (Dagley, 1972b).

This relative metabolic advantage of microorganisms over high organisms might be attributed to the difference in their evolutionary processes. Microorganisms, for the most part, reproduce more rapidly than high organisms and have a high rate of mutation, thus allowing them to develop enzyme systems over the years that will metabolize a greater variety of organic structures; however, the diversity in microbial communities which provides a multitude of possible biochemical pathways is probably even more important.

2. Metabolic Activity

The amount of material consumed by any given group of organisms will depend largely on the product of their biomass and metabolic rate/unit mass. In general, the rate of metabolism increases with the increasing surface/volume ratio (Thimann, 1966).

Organism	Surface/volume ratio
200-lb. man	0.3
Hen's egg	1.5
Amoeba	400
Bacteria (0.5 μ or less)	120,000

The bacteria and fungi may thus be expected to be immensely more metabolically active per unit weight than higher life forms. In one hour, for example, a lactose-fermenting bacterium will break down from 1000 to 10,000 times its own weight in lactose, whereas a man would require 250,000 hours to break down 1000 times his own weight in sugar (Thimann, 1966).

An analysis of the metabolic activity of different organisms in soil by MacFadyen (1957) demonstrates the importance of bacteria in a limited ecosystem (see Figure II-1). The data indicate that bacteria alone account for the major portions of the total metabolism in the soil. This can be attributed to a much larger magnitude of biomass and higher metabolic rates of soil microorganisms compared to soil animals (Table II-1) (MacFadyen, 1963). Dagley (1972b) has also estimated that microbial biomass (including algae) is much greater than that of the combined animal biomass.

This approach of analyzing ecosystems on the basis of metabolic activity is particularly relevant to an assessment of the relative importance of various taxa in that it illustrates the relative rates of energy consumption, which is related to organic material consumption.

3. Availability of Synthetic Organics to Various Taxa

Even given the high order of metabolic activity and versatility shown by the bacteria and fungi, their role in biological degradation might be somewhat limited if significant amounts of synthetic organic chemicals were stored by higher organisms, thus making the chemicals unavailable for microbial degradation; however, such a situation does not seem likely to exist in the environment. For example, Edwards (1973) has calculated the amount of organochlorine insecticides that is locked up in the soil biota. The calculations are based on the average figure of 1.0 ppm of organochlorine residue in the living organisms. Considering that there is an average of 25 tons of living organisms per ha (Stockli, 1950), the amount stored in the biota will be equivalent to 0.025 Kg/ha (0.022 pounds/acre) (Edwards, 1973), which is in the range of 1 to 2% of total (of many herbicides) normally applied per acre (application rate: 1 to 2 pounds actual/acre, Thomson, 1967). DDT may be typical of this group of insecticides. Woodwell and coworkers (1971) have calculated that the total biota in oceans, freshwater and soil probably contains 3.3% or less of the amount of DDT produced in one year (during the mid-1960s). Compatable with this

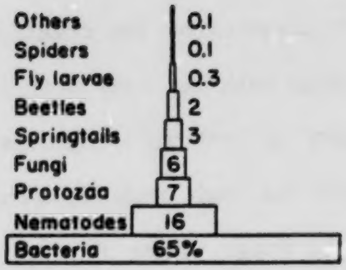

Figure II-1. Percent Total Metabolism by Different Taxa of a Hypothetical Soil Community in a Meadow (Macfadyen, 1957)

Table II-1. Figures From a Hypothetical Grasslands Soil Community Showing Order of Magnitude of Numbers, Biomass, and Metabolism for the Main Biological Groups (Macfadyen, 1963)

Group of Organisms	Weight of Organism (mg)	Approximate estimates grassland per sq m		Metabolism Calories per day per g at 16°C
		Numbers	Mass (g)	
Bacteria	10^{-9}	10^{15}	1000	575
Fungi			400	161
Protozoa	0.05	5×10^{8}	38	14
Nematoda	0.001	10^{7}	1	144
Lumbricidae	5000	10^{3}	120	7
Enchytracidae	0.14	10^{5}	12	100
Mollusca	1500	50	10	29
Myriapoda	25	500	12.5	36
Isopoda	22.7	500	5	36
Opiliones	18	40	0.4	53
Acari-				
Parasitids	0.2	5×10^{3}	1.0	280
Oribatei	0.25	2×10^{5}	2.0	72
Araneae	10	600	6.0	27
Coleoptera	250	100	1.0	39
Diptera	610	200	1.0	29
Collembola	0.46	5×10^{4}	5.0	144

projection, the Study of Critical Environmental Programs (SCEP, 1970) estimates that of the 500,000 tons of DDT existing in the world's oceans, only 0.12% is stored by fish. Although the data presently available is somewhat fragmentary, it does suggest that synthetic organic chemicals that are not readily degradable by higher life forms are also not stored in significant amounts in higher life forms and are thus available for microbial degradation.

Thus, the bacteria and fungi seem to serve and will probably continue to serve as the major factors in the ultimate biodegradation of synthetic organic chemicals in the environment. Consequently, this review has focused on techniques that use, for the most part, microorganisms as the biological agents of degradation.

B. Photochemical Degradation

The photolysis of a chemical in the environment appears to be an important process that may have a considerable effect on a chemical's persistence and degradation. This is especially true of atmospheric contaminants (Altshuller and Bufalini, 1971; Leighton, 1961) and many organic pesticides (Crosby and Li, 1969; Crosby 1969a, 1969b; Plimmer, 1970).

In order for a chemical to react photochemically, it must be able to derive energy from the incident light available to it. In the environment, sunlight is the incident light source. The ozone in the atmosphere effectively absorbs all sunlight of wavelengths <290 nm (Koller, 1965) and for practical purposes, the energy available for photochemical transformations does not exceed 95 kcal/mole (300 nm) (Crosby and Li, 1969). As the wavelength increases, the energy available for photochemical change is reduced. Crosby and Li (1969) have suggested that wavelengths longer than 450 nm (65 kcal/mole) would not be expected to bring about chemical changes under most circumstances (see

Figure II-2). Thus, in terms of environmental photolysis, processes that take place upon irradiation with light between 300 to 450 nm are the most significant.

The theory of how a chemical absorbs and chemically uses the energy from light has been reviewed adequately elsewhere (Calvert and Pitts, 1966; Owen, 1971); however, some very basic fundamentals will be reviewed briefly in order to provide perspective to the experimental techniques that will be discussed.

A molecule may become excited by direct absorption of light or by accepting energy from an excited donor molecule (sensitizer). The ultraviolet absorption spectra provides an indication of the ability of the molecule to absorb energy at various wavelengths (extinction coefficient), and that is dependent upon whether the transition is "allowed" (determined by selection rules, see Jaffe and Orchin, 1962) or "forbidden." This absorption is very dependent upon the matrix of the molecule (vapor phase, absorbed on a solid, dissolved in various liquid). In fact, it is quite possible that a compound that does not absorb at >290 nm in a solvent such as hexane, may absorb light in the environment. For example, Plimmer (1972b) has noted a 60 nm red shift for trifluralin (a nitroaromatic herbicide) when it was adsorbed on silica gel. Sensitization occurs when an acceptor molecule has an excited state of correct energy relative to a donor molecule and it requires some interaction between the donor and acceptor.

Once the molecule is excited, it can release its energy in several non-chemical ways (Figure II-3) including fluorescence and phosphorescence (irradiative decay) and radiationless decay (energy transferred to the matrix). On the other hand, the energy may be used to chemically alter the molecule. The efficiency of each of these processes (usually stated as quantum yield) is again somewhat dependent upon the matrix.

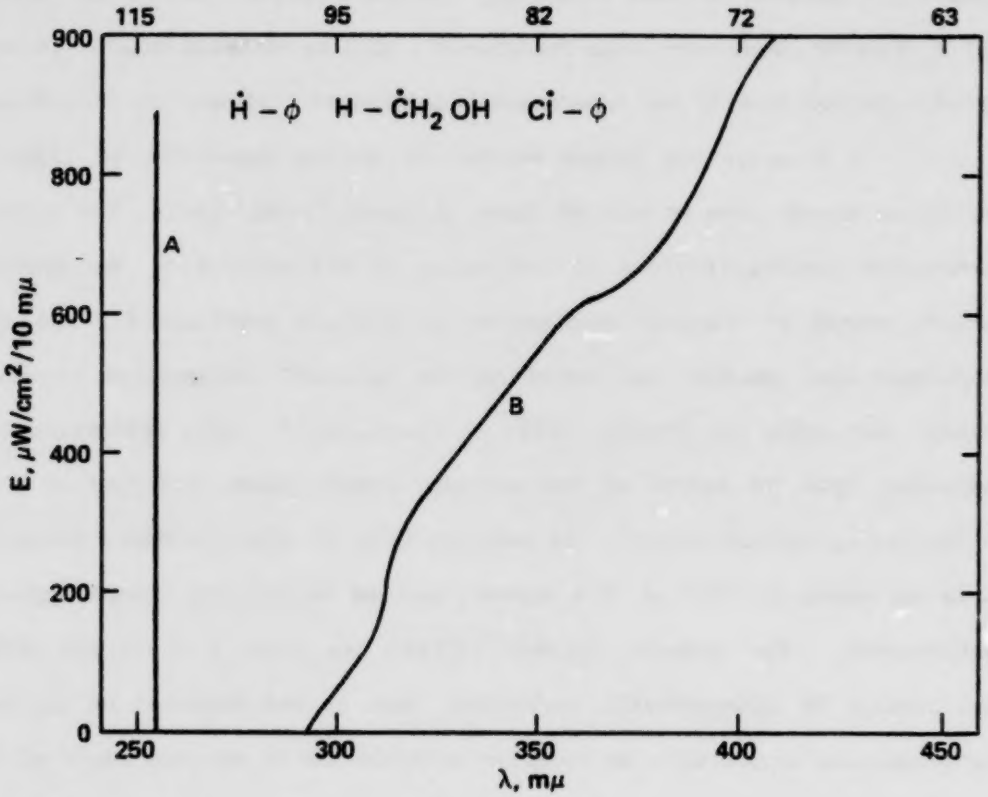

Figure II-2. Spectral Energy of Sunlight (B) Relative to Chemical Bond Energies and Low-Pressure Mercury Arc Lamp (A) (modified from Crosby, 1969b and Crosby, 1972a)

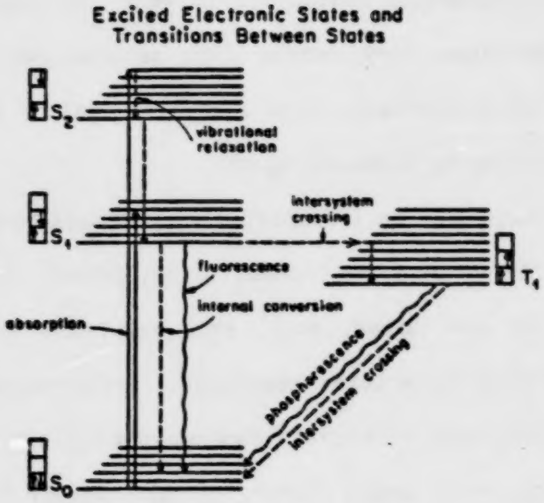
Excited Electronic States and Transitions Between States
S_2
vibrational relaxation
S_1
intersystem crossing
T_1
fluorescence
internal conversion
absorption
phosphorescence
intersystem crossing
S_0

Figure II-3. Modes of Formation and Deactivation of Molecules in the Excited State (Owen, 1971). Reprinted from Organic Chemicals in Aquatic Environments, by courtesy of Marcel Dekker, Inc.

Photochemical alteration can also occur by the interaction of the compound of interest with a photochemically excited molecule (e.g., singlet oxygen - Plimmer, 1972a, Crosby, 1972b; or diethylaniline with DDT - Miller and Narang, 1970). Again the neighboring molecules are extremely important to the process.

Because of these effects which are induced by the matrix, laboratory simulation of environmental photolysis is very difficult. The conditions that are used are sometimes very remote from natural conditions and the results obtained should be extrapolated with a greater deal of caution.

C. Degradation by Chemical Agents

Other nonmetabolic decomposition processes besides photolysis may also be important in the environment (Rosen, 1972a; Crosby, 1969a, 1970). Ubiquitous substances such as air, water, soil, etc. may react with or catalyze environmental degradation or alteration reactions. Unfortunately, with the exception of hydrolysis processes, relatively few non-photolytic chemical (as opposed to biological) reactions are known (Rosen, 1972a). Crosby (1970) has suggested that this low number of reported chemical reactions, at least in soil, is due to a lack of distinction between biological and nonbiological reactions in experimental studies. Such differentiation is extremely difficult because of the difficulty of sterilizing soil without changing the soil structure and chemical composition. In contrast, this distinction between the two processes (biological and chemical) is extremely simple in reactions that take place in the atmosphere since biological processes are minimal. Some of the major non-photolytic chemical processes in the environment will be reviewed briefly.

The free-radical character and solubility in water make oxygen a most effective environmental reactant (Crosby, 1970). Many compounds upon exposure to air will be readily oxidized. In many cases the presence of light accelerates the oxidation process but a considerable number of reactions can take place in the dark (Crosby, 1969a).

Pesticides such as phosphites, sulfoxides and dithiocarbamates are readily oxidized to phosphates, sulfones and thiuram disulfides, respectively. Oxidation reactions are particularly facilitated in the atmosphere (Haagen-Smit and Wayne, 1968) where the medium consists of 20% oxygen. Other oxidation reactions initiated by oxidants or free radicals formed in photochemical smog may also be important to the environmental fate of a chemical substance (Crosby, 1969a) and oxidation by hydroxyl and alkylperoxy radicals in water has received some study recently (Mill _et al_., 1979, 1980).

Water, besides being an important medium for chemical reactions, is reactive itself through its ability to form either hydroxyl or hydrogen ions. Consequently, pH becomes important in nonmetabolic decomposition of chemicals in an aqueous medium. Normally, drinking water pH is between 5 and 8, although values as high as 10.5 have been noted (Crosby, 1969a). In soil, the water is seldom neutral and can vary from a pH of 3 to 1.5 (Crosby, 1970). Although solvolysis and elimination reactions have been noted (Rosen, 1972a; Crosby, 1969a), the most widely recognized and studied reaction with water is hydrolysis. The reaction is usually pH dependent and can be catalyzed by various agents (e.g., hydrolysis of parathion is catalyzed by common amino acids, hydroxylamine derivatives, metal ions, and metal ion chelates). With organophosphorus esters, the hydrolysis reaction mechanism is different under acidic or neutral conditions (alkyl-oxygen bond is attacked) compared to under alkaline conditions (phosphorus-oxygen bond attached).

Many chemical substances that enter the environment become associated with soil and dust particles. Some mineral constituents of soil, such as clays, can act as catalysts. For example, they can cause endrin to isomerize from an epoxide to a ketone. The organic constituents of soil can serve as oxidizing and reducing agents since they contain a relatively high concentration of stable organic free radicals.

All of the above processes can be accelerated by an increase in temperature and, therefore, temperature is an important degradation parameter.

In general, non-photolytic reactions affected by chemical agents have received considerably less attention than other environmental processes; however, it should be kept in mind that in many systems used to study biological or photochemical degradation, degradation by chemical agents cannot be excluded.

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III. BIODEGRADATION OF CHEMICALS IN AQUATIC OR SEWAGE TREATMENT CONDITIONS

Organic chemicals that enter the aquatic environment can be degraded by chemical or biological agents. Techniques which are used to study degradation processes that are affected by chemical agents are reviewed in Section V. In this section, the biodegradation techniques which have been used since 1975 are reviewed. The section has been divided into two major subdivisions; (1) static tests which may be used to simulate natural water systems or wastewater treatment conditions (depends upon acclimation, microorganism concentration, nutrient sources, etc.), and (2) dynamic (semi-continuous or continuous) systems which usually maintain high concentrations of microorganisms and thus more closely simulate conditions of a biological wastewater treatment plant.

Since the 1975 report, a large number of biodegradation tests have been proposed and required by U.S. and foreign regulatory agencies and many of these protocols have been or are in the process of being evaluated and validated. Table III-1 provides a summary of these tests along with some of the characteristics of the tests. The information on the tests being evaluated by the Organization for Economic Cooperation and Development (OECD) was taken from an article by Gerike and Fischer (1979). A later article by Gerike and Fischer (1981) was not available in time for it to be reviewed in detail. However, some of the conclusions are mentioned. The individual tests will be discussed in detail in the following sections.

A. Static Aqueous Biodegradation Techniques

As indicated in our earlier report, biodegradation is generally measured in static systems by exposing the test compound to microorganisms and analyzing periodically for the disappearance of the intact test compound, the formation of degradation products, or the consumption or evolution of an indirect

Table III-1. Characteristics of Biodegradability Tests Which are Currently Being Evaluated

Pesticide Proposed Guidelines - U.S. EPA (1978a)

Test	Model for	Principle	Inoculation	Conc. Test Chemical (mg C/liter)	Duration (days)	Analysis
Aerobic Aquatic Metabolism Krzeminski et al. (1975) Schaefer and Dupras (1976)	Surface water	Die-away natural water	Natural Sample		< 30	^{14}C
Anaerobic Aquatic Metabolism Gowda and Sethunathan (1976) Hance and Chesters (1969) Miyazaki et al. (1975) Sikka and Rice (1973)	Water sediments	Die-away water plus sediment or flooded soil	Natural sample	Approximate field rate	< 365	^{14}C
Effect of Microbes on Pesticides Benezet and Matsumura (1974) Furukawa and Matsumura (1976) Sikka and Saxena (1973) VanAlfen and Kosuge (1974)	Microbial degradation	Die-away pure or mixed cultures	Water or soil	--	< 30	^{14}C or direct analysis (GC, etc.)
Activated Sludge Metabolism Krzeminski et al. (1975) SDA (1965)	Communal sewage treatment plant	Repeated batch die-away mineral and organic nutrients	Sludge from sewage treatment plant - maintained at 2500 ± 500 mg/l MLSS	0.1-100	Variable	^{14}C

Table III-1. Characteristics of Biodegradability Tests Which are Currently Being Evaluated (Cont'd)

U.S. Toxic Substances Control Act Section 5
Guidelines for Premanufacturing Notices, U.S. EPA (1979)

Test	Model for	Principle	Inoculation	Conc. Test Chemical (mg C/liter)	Duration (days)	Analysis
Shake Flask Method SDA (1965) and Modified OECD Screening Test	Surface water	Die-away mineral nutrients	Soil/sludge Effluent after acclimation (1 ml/liter)	10	21	DOC CO_2 optional
Activated Sludge SDA (1965) - SCAS Zahn and Wellens (1974) Zahn and Huber (1975)	Communal sewage treatment plant	Repeated batch die-away mineral and organic nutrients	Acclimated effluent (SCAS) sludge 500 mg/l	50-100	19	DOC
Anaerobic Digestion Nottingham and Hungate (1969)	Sewage treatment plant digestors	Die-away mineral nutrients	Anaerobic digestor sludge	200	28	CH_4 CO_2
Carbon Dioxide Evolution Sturm (1973)	Surface water	Die-away mineral nutrients	Composite effluent after acclimation	5-10	28	CO_2
Biochemical Oxygen Demand	Surface water	Die-away mineral nutrients	Effluent after acclimation (0.1-0.5 ml/liter)	1-10	28	BOD

Table III-1. Characteristics of Biodegradability Tests Which are Currently Being Evaluated (Cont'd)

Tests Being Evaluated by OECD

Test	Model for	Principle	Inoculation	Conc. Test Chemical (mg C/liter)	Duration (days)	Analysis
Closed Bottle Test Fischer (1975)	Surface water	Die-away mineral nutrients	1 drop of effluent/liter	≈1	30	BOD
Modified OECD Screening Test	Surface water	Die-away mineral nutrients	0.05 percent effluent	3-20	19	DOC
Sturm (1973) Test	Surface water	Die-away mineral nutrients	Effluent after acclimation	≈10	28	CO_2 DOC
French AFNOR Test	Polluted river	Die-away mineral nutrients	$(5 \pm 3) \times 10^5$ germs/ml	40	42	DOC
Japanese MITI	Sewage treatment plant	Die-away mineral nutrients	30 mg sludge/liter	-50	14	DOC BOD
Swiss EMPA Test (used instead of Zahn-Wellens for surfactants)	Industrial sewage treatment plant	Die-away mineral nutrients	2.0 or 0.2g sludge/liter	≈50	14	DOC
Zahn-Wallens Test	Industrial sewage treatment plant	Die-away mineral nutrients	1g sludge/liter	≈400	14	DOC COD
Coupled Units OECD Confirmation Test	Communal sewage treatment plant	Steady state organic nutrients	Effluent or 2.5g sludge/liter	≥12	Variable	DOC

Table III-1. Characteristics of Biodegradability Tests Which are Currently Being Evaluated (Cont'd)

Tests Being Considered by ASTM

Test	Model for	Principle	Inoculation	Conc. Test Chemical (mg C/liter)	Duration (days)	Analysis
Aerobic Ultimate Biodegradability Gledhill (1975a)	Surface water	Die-away mineral nutrients	Soil/sludge effluent after acclimation	10	28	CO_2 DOC
Anaerobic Biodegradability Owen *et al*. (1979) Healy and Young (1979)	Sewage treatment plant digestors	Die-away mineral nutrients	Anaerobic digestor supernatant	50	28	CO_2/CH_4 Pressure

parameter such as oxygen or carbon dioxide. Thus, three components are essential to an organic chemical biodegradation test system: the chemical compound, the biological test media, and the analytical method. The type and the source of the inoculum and the conditions used in the test method are among the important factors that influence biodegradation. Classification of the test methods in this section has been aided by consideration of these factors. In our previous report, we organized the test methods both by the test media and by analytical method (e.g., biochemical oxygen demand), the latter being used in instances where the method had been so widely used that it is more or less regarded as a biodegradability test method. However, in the newer literature, a variety of analytical methods may be used in the same test procedure. As a result, in this report the analytical methods have not been used for organization of the test methods. Also the distinction of shake culture (as opposed to non-shaking cultures) does not seem to be worthwhile. In this report we have organized the static biodegradability tests into five categories: (1) Mineral Salts-Dilute Microbial Concentration -- mineral salts media with or without external carbon with a low concentration of microbial cells, (2) Mineral Salts-High Microbial Concentration -- defined mineral salts media with or without external carbon with a high concentration of microbial cells, (3) Natural Aqueous Systems -- undefined nutrient media such as river water, water and hydrasoil, or microcosm studies, (4) Anaerobic Media, and (5) Pure Culture Studies.

1. Mineral Salts-Dilute Microbial Concentration

a. Standard BOD Test

The standard BOD dilution test continues to be used by many investigators. For example, Bridie et al. (1979) have used the standard 5 day BOD test with approximately 90 commercial chemicals. The only deviation from the APHA "Standard Method" procedure was the addition of 0.5 mg/ℓ of allylthiourea in each test to prevent nitrification. Each 500 ml test solution was seeded with 10 ml of filtered effluent from a biological sanitary waste treatment plant that in some instances was adapted to the chemical. With sparingly soluble chemicals, magnetic stirring was incorporated.

Price et al. (1974) used the standard BOD procedure (run for 20 days) on 50 synthetic organic compounds using three concentrations (3, 7, and 10 mg/ℓ). They analyzed nitrites and nitrates during the test to determine oxygen demand due to oxidation of ammonia nitrogen and organic nitrogen, but did not add nitrification inhibitors because they cited literature that suggested inhibitors adversely affected biodegradation. For compounds that did not degrade readily, acclimated inoculum was used. These authors also tested the more biodegradable compounds in a synthetic seawater BOD test. The test media consisted of the nutrient salts and buffers of BOD dilution water added to artificial seawater. The seed that was used was maintained by adding small amounts of settled raw wastewater about every 3 or 4 days to seawater taken from Lavaca Bay, Texas.

Subba-Rao and Alexander (1977) also used the standard BOD test with nine analogs of DDT. The chemical was introduced as the sole source of carbon at a concentration of 2 mg of carbon per bottle. The chemicals were added in acetone solution and 5 mg of Hudson Collamer silt loam was used as the source of microbial inoculum. A slightly different inorganic salts solution was used instead of standard BOD water. The dissolved oxygen was measured at 5, 10, 20, 30, and 40 days with an oxygen analyzer and nitrite and nitrate levels were also measured (not evident until day 40). Also, attempts were made to isolate microorganisms from the BOD bottles using enrichment culture technique with 0.1 percent of the test chemical as the sole source of carbon. Isolation of bacteria that could utilize the chemical was considered to be a positive indication of biodegradation even if the oxygen consumption was low.

b. MITI Biodegradation Test (MITI, 1977; Kubota, 1979; Sasaki, 1978)

In 1974, the Japanese "Law Concerning the Examination and Regulation of Manufacturing, etc., of Chemical Substances" came into effect. Among other requirements, this law requires that the Minister of International Trade and Industry (MITI) along with the Minister of Health and Welfare prescribe "tests on the degradability of the chemical substance by microorganisms, etc., for the decision whether the chemical substance has the property of persistence against natural effects."

The test method required by the MITI is basically a closed system oxygen consumption test although other indirect and direct analyses are also used. Considerable effort is expended in order to obtain diverse inoculum for the activated sludge that is used for inoculum in the test (MITI, 1977). One liter of return sludge, one liter of surface water, and one liter of surface soil is obtained four times a year at not less than 10 places throughout the country. These samples are stirred in one container, allowed to stand, filtered through No. 2 filter paper, and adjusted to pH 7.0. After aeration for 30 minutes, 1/3 of the supernatant is removed and an equal volume of 0.1 percent synthetic sewage (1 gram each of glucose, peptones, and monopotassium phosphate in 1 liter of water) is added and the procedure is repeated every day.

Four test vessels are prepared: (1) mineral salts media with 100 ppm (w/v) of test compound, (2) control blank containing only mineral salts media, (3) water with no inoculum to which is added 100 ppm (w/v) of the test compound (chemical degradation control blank), and (4) mineral salts media to which is added 100 ppm (w/v) aniline. Inoculum is added so that the concentration of suspended matter is 30 ppm (w/v). After inoculation, all test vessels are incubated at 25°C for a usual period of two weeks and during that time changes in oxygen consumption are measured. In addition, residue amounts of the test compound are determined. If the degradation of aniline does not exceed 40 percent of the calculated total oxygen consumption, the test is considered invalid. A variety of closed system oxygen consumption measuring apparatus could be used. Gerike and Fischer (1979) used a Sapromat (BOD determining apparatus with electrolytic oxygen supply) but indicate that a Warburg apparatus or a closed bottle test with an overhead gas phase could be used. The apparatus used by the Chemical Biotesting Center (CBC), Chemical Inspection and Testing Association in Tokyo, Japan (Kitano, 1978) is depicted in Figure III-1 and is similar in operation to the Sapromat (see Figure 7, p. 67, Howard *et al*., 1975). Volatile chemicals have been handled in the CBC apparatus by placing a capillary tube between the culture vessel and the electrolysis flask and water insoluble substances are decomposed satisfactorily within the test period of two weeks when pulverized before testing (Kitano, 1978).

Yoshida and Kojima (1978) have used the MITI test with radiolabeled compound to evaluate the biodegradation of diisopropylnaphthalene. Inui *et al*. (1979) also used radiolabeled compound in the study of the biodegradation of 2,6-di-*t*-butyl-4-methylphenol (BHT) with the MITI test. However, the latter authors also varied the test chemical concentration (0.3-30 ppm) and activated sludge concentration (1-100 ppm).

c. Closed Bottle Test (Fischer *et al*., 1974; OECD, 1979a)

The Closed Bottle Test of Fischer is a 28-day BOD test. Inoculum can consist of soil (100 g unfertilized garden soil in 1 liter of chlorine-free tap water, dispersed for 30 minutes, suspension filtered through coarse filter, first 200 ml filtrate discarded, remaining filtrate is inoculum - 1 drop/liter - should not exceed 10^3 to 10^5 organisms/ml of final volume), effluent from an activated sludge plant or trickling filter (filter through coarse filter, first 200 ml filtrate discarded, remaining filtrate is inoculum)

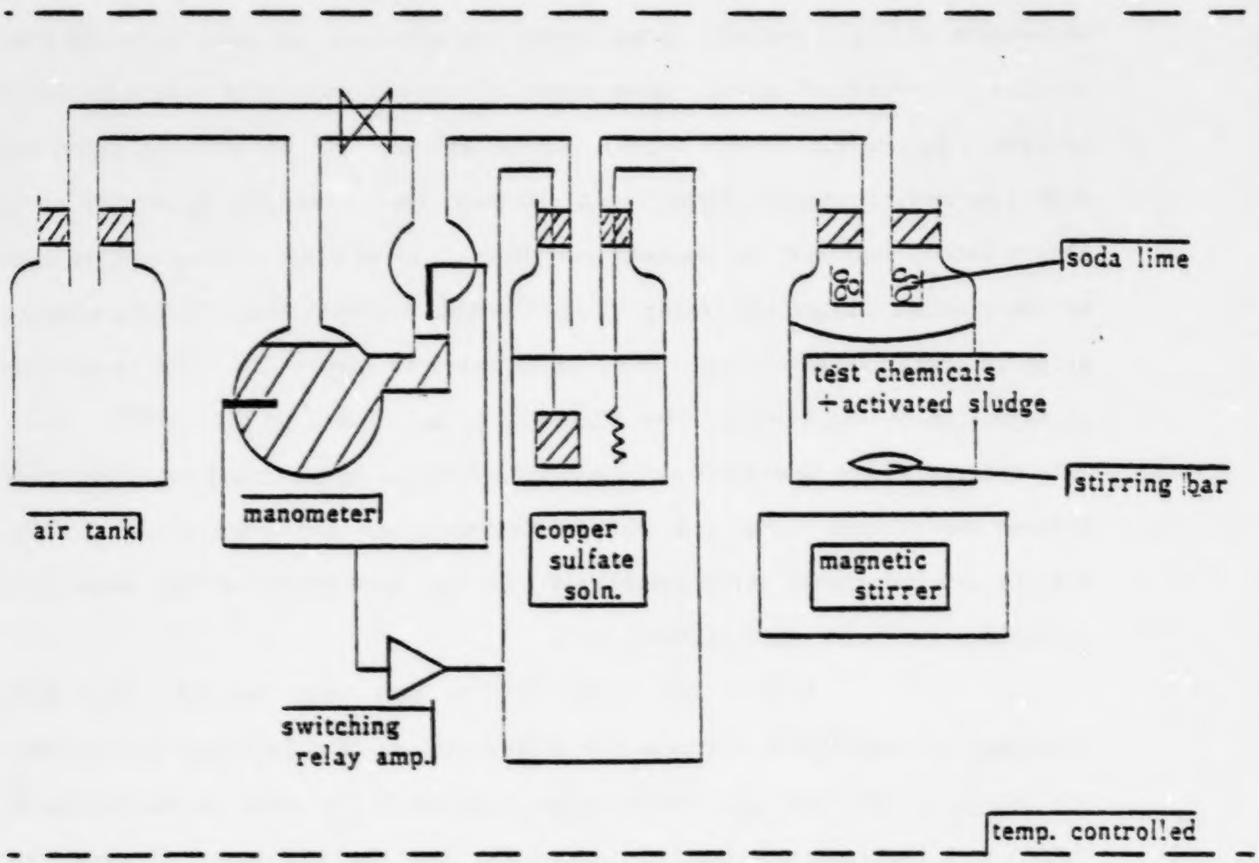
soda lime
test chemicals
+ activated sludge
stirring bar
air tank
manometer
copper
sulfate
soln.
magnetic
stirrer
switching
relay amp.
temp. controlled

Figure III-1. Closed System Oxygen Consumption Measuring Apparatus (Kitano, 1978)

or effluent from a strongly aerated laboratory activated sludge plant. The bottles are treated in the scheme indicated in Figure III-2. The large bottles are first filled with distilled water and mineral salts and then the test and control materials are added to give final concentrations of 2 mg/ℓ and sometimes 5 or 10 mg/ℓ (poorly degradable compounds). One drop of inoculum per liter of final volume is added to the test and control determinations and to the inoculum blank. Calibration compounds are sodium n-dodecylsulfate (should achieve $\geq$ 40 percent $BODT_{28}$) and linear alkylbenzenesulfonic acid sodium salt (should achieve $\geq$ 40 percent $BODT_{28}$). The oxygen depletion without inoculation should not exceed 0.2 to 0.3 $mgO_2/ℓ$ after 5 days and 0.4 $mgO_2/ℓ$ after 28 days and the blank with inoculation should not exceed 0.4 to 0.5 $mgO_2/ℓ$ after 5 days and 0.5 to 0.6 $mgO_2/ℓ$ after 15 to 28 days. An inhibition test is suggested if illogical results are obtained. When Gerike and Fischer (1979) used the Closed Bottle Method with nitrogen-containing compounds, they calculated the theoretical BOD with the assumption that the final oxidation state of nitrogen would be -3 (NH_3) unless the starting material was a nitro compound, in which case it was assumed to be +5 (NO_3).

Fischer (1975) has also modified the Closed Bottle Test to allow for analysis by other methods than BOD determination. The oxygen concentration which can be achieved in the conventional Closed Bottle Test (approximately 9 ppm) only allows 2 to 5 ppm of the test compound to be used. These small concentrations of the test chemical preclude use of analytical methods such as COD, TOC, DOC, and CO_2 for determining degradation. However, by using pure oxygen, an oxygen saturation of approximately 35 ppm O_2 is possible and test material concentrations up to 30 ppm can be used. Fischer (1975) indicated that the degradation kinetics with the compounds they have examined are basically the same for the air saturated version compared to the pure oxygen

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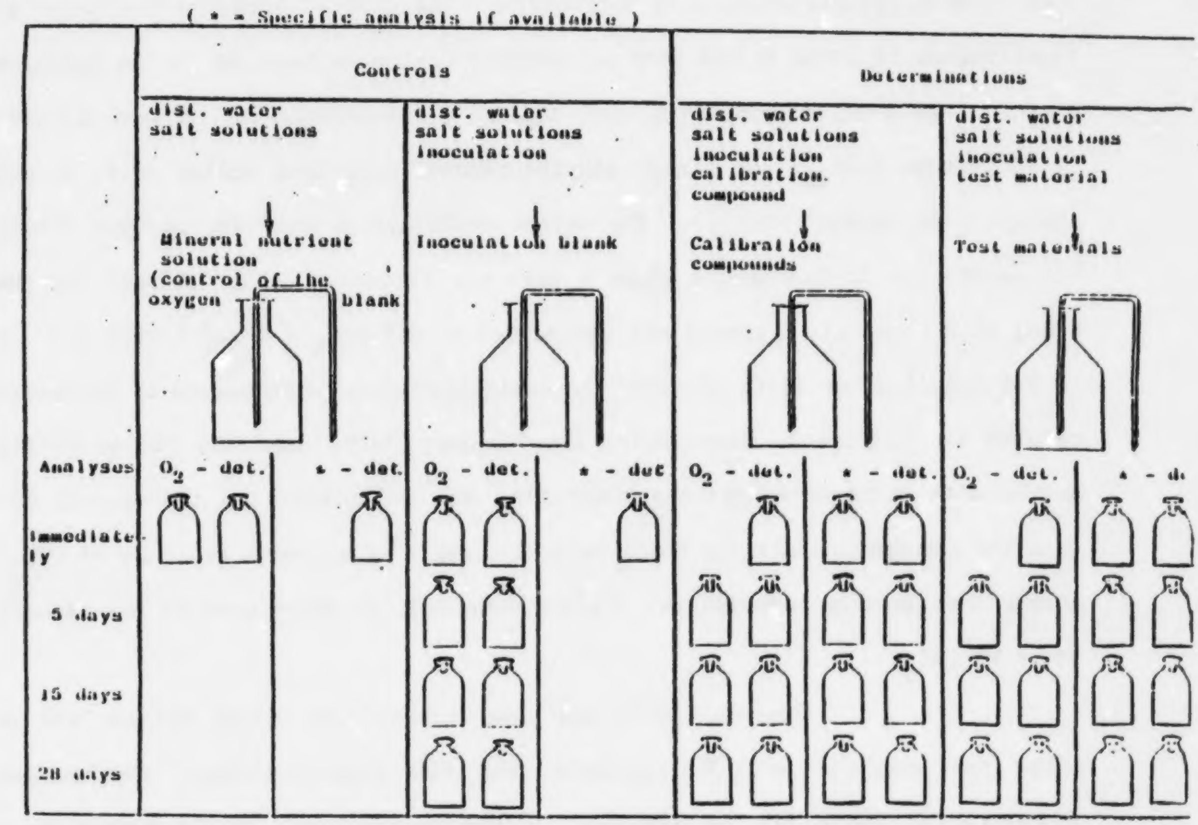
(* = Specific analysis if available)
Controls
Determinations
dist. water
salt solutions
Mineral nutrient
solution
(control of the
oxygen blank
dist. water
salt solutions
inoculation
Inoculation blank
dist. water
salt solutions
inoculation
calibration
compound
Calibration
compounds
dist. water
salt solutions
inoculation
test material
Test materials
Analyses
O_2 - det.
* - det.
Immediately
5 days
15 days
28 days

Figure III-2. Scheme for bottle arrangement for the closed bottle test (OECD, 1979a).

version. However, it is possible that in some situations, pure oxygen may inhibit the degrading microorganisms.

d. Sturm CO_2 Evolution Test (Sturm, 1973; OECD, 1979b)

The Sturm CO_2 Evolution Test has its beginning with a biodegradability study of some organic cyanides in river water by Ludzack and Ettinger (1963) where the results were expressed in terms of cumulative CO_2 recovered as a percent of theoretical achievable. In a study of NTA, Thompson and Duthie (1968) of Proctor and Gamble Company modified the procedure, (1) by using BOD dilution water instead of river water and (2) by adding a Bunch-Chambers 14 day die-away with no transfer as an acclimation period for the sewage-derived bacteria. Sturm (1973), who is also from Proctor and Gamble Company, further modified the test by scaling it down from 20 to 6 liters and used a BOD Test with the CO_2 production method, although he stated that the CO_2 test was thought to be more reliable.

The Sturm CO_2 Evolution Test (Sturm, 1973) has been run with eight materials and a positive control (see Figure III-3), although any number of chemicals can be used. The acclimation cultures (see composition in Figure III-3) are allowed to sit quiescently in the dark for 14 days after which 700 ml of each is used to make a composite seed. For the CO_2 test, each carboy includes 600 ml of composite seed and 120 mg of test material brought to 6 liters with BOD water. During the test period of 26 days, CO_2-free air is bubbled through the test unit and evolved CO_2 is collected with barium hydroxide. At day 25, the test vessels are acidified to pH 3.0 in order to release any CO_2 that may be trapped in the media. Dextrose is used as a positive control and should exceed 80 percent of theoretical for the test to be valid. OECD (1979b) suggests that dextrose should normally produce over 70 percent theoretical CO_2 in 10 days.

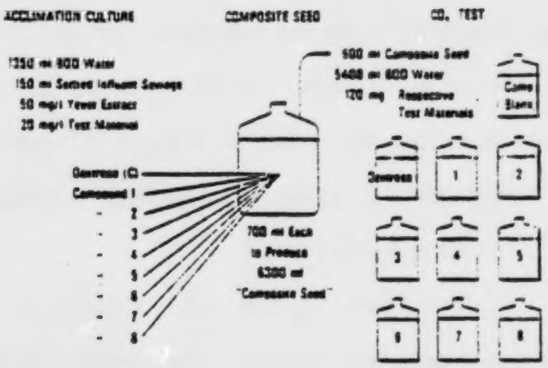
ACCLIMATION CULTURE
COMPOSITE SEED
CO₂ TEST
1350 ml BOD Water
150 ml Settled Influent Sewage
50 mg/l Yeast Extract
20 mg/l Test Material
Glucose (C)
Compound 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
700 ml Each to Produce 6300 ml "Composite Seed"
900 ml Composite Seed
5400 ml BOD Water
120 mg Respective Test Materials
Comp Blank
Glucose
1
2
3
4
5
6
7
8

Figure III-3. General protocol for 10 unit Sturm CO_2 evolution test (Sturm, 1973)

Saeger and Tucker (1976) have used the Sturm CO_2 test to study the biodegradation of phthalic acid esters (PAE). The activated sludge supernatant for the acclimation culture was already acclimated to one PAE. The procedure was also used by Saeger *et al*. (1979) to evaluate the biodegradation of aryl phosphates with settled supernatant from a semi-continuous activated sludge laboratory unit used as seed.

The OECD (1979b) protocol for the Sturm CO_2 test is slightly different in the development of the seed. Two liters instead of 1.5 liters of acclimation culture are prepared with the same proportions used by Sturm (1973). The 2 liters of acclimation culture are incubated in a dark room for 14 days on a rotary shaker (100 ± 10 oscillations/min). After the 14 days, each 2 liter acclimation culture is filtered through glass wool to remove large biological floc and slime particles and the filtrate is diluted 1:10 in the CO_2 test medium. No composite acclimated seed is developed. Also, to each test carboy (1 gallon) only a total of 3 liters of medium and seed are prepared instead of the 6 liters in the original Sturm test. The OECD (1979b) test guideline also allows an alternative procedure for preacclimation which used the SDA laboratory semi-continuous activated sludge (SCAS) unit to develop the seed as is suggested by Larson (1979) and has been used by Saeger *et al*. (1979). However, Saeger *et al*. (1979) mixed 100 ml of the supernatant from the SCAS unit with 900 ml standard BOD water whereas the OECD (1979b) procedure indicates that a 1 percent homogenized inoculum for each CO_2 test flask should be used. Larson (1979) has indicated that a 1 percent (vol/vol) inoculum from a SCAS unit is "sufficient to achieve reproducible population densities typical of surface waters" (10^4 to 10^5 colony-forming units/ml). The difference is explained by the homogenization step which increases the microbial concentration 20-fold.

Gledhill (1978) also used the standard Sturm CO_2 test as a biodegradability screening method for a new detergent builder, carboxymethyltartronate.

e. Larson CO_2 Evolution Test (Larson, 1979)

Another Proctor and Gamble Company employee (Larson, 1979) has developed a CO_2 evolution test that is meant to be used with a semi-continuous activated sludge (SCAS) unit for estimating biodegradation potential. The procedure has some significant differences from the Sturm CO_2 test. For water soluble compounds, inocula for the CO_2 evolution test are generated by standard Soap and Detergent Association (SDA) SCAS sludge procedure with the activated sludge exposed to increasing levels of test material (up to 20 mg/ℓ) (removability studied in these units by measuring DOC reduction). Composited samples (100 ml of mixed liquor from two duplicate SCAS units) were homogenized for 2 minutes at medium speed in a Waring blender, settled for 15 to 30 minutes, and 1 percent of the cloudy supernatant was used for inoculum. SCAS removability studies were not conducted with water insoluble compounds since DOC could not be measured. For these compounds, inocula for the CO_2 evolution test were obtained from different sources. One source was activated sludge from a SCAS unit that had not been exposed to any test material. Another source was from settled influent wastewater. Both inocula had similar initial bacterial populations and consisted of 1 percent volume of the CO_2 test volume (10 ml/ℓ).

The test was conducted in a closed shake flask system with the test material as the sole carbon and energy source. The flask contained a final volume of 1 liter made up of the inoculum and a modified BOD water medium (BOD-PLUS-standard BOD water with two times level of phosphate buffer, four times level of $FeCl_2$, and 40 mg of $(NH_4)SO_4$ per liter of high quality water - 18 Mohm - the low level of background carbon and lack of alternate carbon and energy sources simplified DOC and CO_2 measurements). Flasks were aerated with CO_2-free compressed air and the CO_2 that evolved was collected. With water soluble compounds DOC was also measured. Test materials were assayed at replicate concentrations (10 and 20 mg/ℓ) and a glucose control (20 mg/ℓ) and blank were run. Testing was continued until CO_2 production reached a plateau. Statistical analysis of multiple CO_2 evolution experiments were conducted and fit to a "nonlinear computer model which allows the rate and extent of biodegradation for different compounds to be compared" (Larson, 1979). This is described in considerable detail in another article by Larson (1980). In all experiments, a lag period where appropriate metabolic mechanisms or an active population or both are being selected, a period of maximal degradation rate, and a plateau period where a defined extent of metabolism of the molecule is determined were all noted.

Larson (1979) found a 20-fold increase in the number of colony-forming units (CFU) after activated sludge (2500 to 3000 MLSS) was homogenized for 2 minutes and allowed to settle for 30 minutes and this was fairly constant (10^6 to 2×10^7 CFU) for six different batches over an 8 month period. With the homogenization step, a 1 percent (vol/vol) inoculum appears adequate to provide microbial population densities typical of surface waters.

f. Soap and Detergent Association (SDA) Presumptive Test (SDA, 1965; ASTM, 1968)

This was reviewed in our earlier report but since it continues to be used, it will be briefly described here for comparison to other procedures. This method has been standardized by ASTM (1968). In the presumptive test, microorganisms (natural, laboratory, or pure cultures maintained in basal medium plus 30 mg/ℓ of 1-dodecene LAS by weekly transfers of 1 ml of 7 day culture to each 100 ml of fresh medium) are inoculated into a flask that contains a mineral salts and yeast medium (see Table III-2) and the surfactant is tested. The mineral salts ingredients are similar to standard BOD water except all the mineral salts concentrations are considerably higher (see Table III-3). Following two 72-hour adaptive transfers, 1 ml of final inoculum is used per 100 ml of fresh medium and the test is run for 8 days. Biodegradation is determined by measuring the reduction in surfactant content (methylene blue active substances). If the degradation of ABS type compounds equals or exceeds 90 percent, the compound is considered adequately biodegradable by SDA. If degradation falls between 80 and 90 percent, the biodegradation must be confirmed in the SCAS confirmatory test.

g. OECD Screening Test and "Modified" OECD Screening Test (OECD, 1976; OECD, 1979c)

OECD (1976) has developed a static screening test to be applied to anionic and nonionic surfactants. Products are considered acceptable if the biodegradability exceeds the required percentage. If the product biodegradability does not exceed the required percentage, then a dynamic, confirmatory test is required.

The test consists of incubating in a shake flask the surface active substances (5 mg/ℓ) dissolved in nutrient media (BOD dilution water) with an unacclimated inoculum from the secondary effluent (coarse filtered) of a domestic biological sewage treatment plant. Generally, 0.5 mg/ℓ of inoculum is adequate but this should be checked with the standards of biodegradability (Marlon A- should give 92 percent biodegradation; tetrapropylene benzene sulphonate (TBS) - should give 0 to 35 percent). The content of the surfactant is measured periodically until it falls to a constant level (OECD (1976) recommends 5th day and every other day from the 8th day onwards). Anionic

Table III-2. Basal Medium for SDA Presumptive Test (ASTM, 1968)

NH_4Cl	3.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.25 g
KCl	0.25 g
$FeSO_4.7H_2O$	0.002 g
Yeast Extract	0.30 g
Water	1.0 liter

Table III-3. Standard BOD Dilution Water

KH_2PO_4	0.0085 g
K_2HPO_4	0.02175 g
$Na_2HPO_4.7H_2O$	0.0334 g
NH_4Cl	0.0017 g
$MgSO_4.7H_2O$	0.0225 g
$CaCl_2$	0.0275 g
$FeCl_2.6H_2O$	0.000025 g
Water	1 liter

surfactants are determined as methylene blue activated substance (MBAS) and nonionic surfactants are determined as bismuth iodide active substance (BIAS) by the method of Wickbold. The duration of the test should not exceed 19 days.

In the "Modified" OECD Screening Test (OECD, 1980), conditions have been changed to allow for DOC analysis and the time of the test has been extended to 28 days. Also, trace elements and vitamins are added to the nutrient media. Hydroquinone at a concentration of 20 mg DOC/liter is used as the calibration chemical (should exhibit DOC-removal of $\geq$ 60 percent within 28 days). The test compound is run at a concentration of 5 to 40 mg DOC/liter. A control with inoculum but without the test compound is run parallel for determining DOC blanks. DOC is determined with samples that are membrane filtered or centrifuged before carbon determination. The ammonium concentration specified in the OECD Screening Test is tripled to maintain an optimum C:N:P ratio (Gerike and Fischer, 1979; OECD, 1979c, 1980). In addition, a trace metal and essential vitamin solution were added in order to optimize test conditions (Gerike and Fischer, 1979; OECD, 1979c). Although the latest version of the "Modified" OECD Screening Test (OECD, 1979c) recommends a test duration of 28 days, Gerike and Fischer (1979) only ran it for 19 days when they compared the method to six others with a large number of compounds. OECD (1979c) allows inoculum to be developed from soil or surface waters as well as from secondary effluent as long as the suitability of the inoculum is checked with the hydroquinone standard.

Leidner _et_ _al_. (1980) used the OECD Screening Test to study the influence of sulphonic acid group on the biodegradability of _n_-alkylbenzene sulphonates (LAS). For inoculum, they used 2 ml of mixed microorganisms prepared by diluting a suspension of garden soil.

h. French AFNOR Test (AFNOR, 1977; Gerike and Fischer, 1979; OECD, 1979d)

For the inoculum for this test, three samples from surface water that is receiving effluent from a community sewage treatment plant but is free of industrial pollution are used and each sample must have a microbial count of at least 10^5 bacteria/ml. The microbes are filtered with a membrane filter and then taken up and mixed well in an isotonic solution for measurement of concentration by absorbance at 620 nm. The absorbance is compared to a standard curve for Pseudomonas fluorescens strain ATCC 15453. Enough volume of test medium (Table III-4) should be added to adjust the concentration of microbes to $5 \pm 3 \times 10^7$/ml.

Seven flasks are prepared with 150 ml in 500 ml conical flasks under sterile conditions; three with 40 mg C/ℓ of test compound, one sterile control, one glucose control, one control of inhibitory action (glucose and test chemical), and one inoculum control. The flasks are incubated on a shaker and DOC is measured at time 0 and at days 3, 7, 14, 28, and 42 by centrifuging a 3-5 ml aliquot from each flask. In order for the test to be valid, (1) the dissolved oxygen on day 3 in the glucose control must be at least 5 mg/ℓ, (2) the level of glucose degradation at day 7 must be at least 80 percent, (3) the sterile control must still be sterile at the end of the test, and (4) the level of glucose degradation in the inhibitory control should be at least 75 percent of the glucose control.

i. Gledhill Shake-Flask CO_2 Evolution Test (Gledhill, 1975a; ASTM, 1979a)

Monsanto Company (Gledhill, 1975a) has also used a CO_2 evolution test that is similar to the Sturm (1973) test except that much smaller volumes (500 ml instead of 9 liters) are used. A specially equipped shake flask apparatus (see Figure III-4) containing 500 ml of culture medium in a 2 liter

Table III-4. AFNOR Test Medium

	g/ℓ of medium
$(NH_4)_2\ SO_4$	0.300
NH_4NO_3	0.150
KH_2PO_4	0.300
$Na_2HPO_4.12H_2O$	2.000
$MgSO_4.7H_2O$	0.050
$CaCl_2.2H_2O$	0.050
Yeast Extract	0.005
$FeSO_4.7H_2O$	0.010
$MnSO_4$	0.010
K_2MoO_4	0.0025
$Na_2B_4O_7.10H_2O$	0.0025
$Co(NO_3)_2.6H_2O$	0.0025
$CuCl_2.2H_2O$	0.0025
$ZnCl_2$	0.0025
NH_4VO_3	0.0010

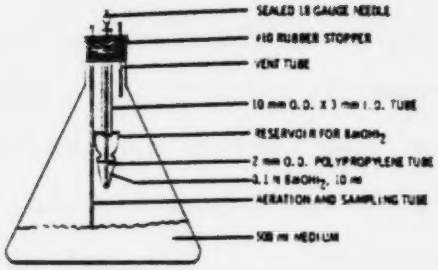
SEALED 18 GAUGE NEEDLE
#10 RUBBER STOPPER
VENT TUBE
10 mm O.D. X 3 mm I.D. TUBE
RESERVOIR FOR Ba(OH)2
2 mm O.D. POLYPROPYLENE TUBE
0.1 N Ba(OH)2, 10 ml
AERATION AND SAMPLING TUBE
500 ml MEDIUM

Figure III-4. Shake flask apparatus for Gledhill CO_2 Evolution Test (Gledhill, 1975a).

Erlenmeyer flask is used in the test. The $Ba(OH)_2$ container (10 ml of 0.2 M $Ba(OH)_2$), which collects the evolved CO_2, was made by attaching a 50 ml heavy duty centrifuge tube to a glass rod and a constriction was placed just above the 10 ml level to prevent spilling of $Ba(OH)_2$ into the medium. The $Ba(OH)_2$ is periodically sampled through a polypropylene tube.

An acclimated inoculum was prepared by inoculating a mineral salts-vitamin medium (Table III-5) supplemented with 25 mg/ℓ each of Difco vitamin-free Casamino Acids and yeast extract, 2.5 g/ℓ of fresh topsoil, and 50 ml/ℓ of freshly settled raw domestic sewage. These ingredients were mixed for 10-15 minutes, screened (24 mesh - 707 μm), and 100 ml volumes added to 300 ml Bellco baffled shake flasks where the test compound was added at concentrations of 10, 20, and 10 mg/ℓ on days 0, 7, and 11, respectively. At day 14, the inocula were pooled and five volumes of inoculum were mixed with one volume of freshly settled raw sewage.

In the test, 60 ml of inoculum were added to 440 ml of mineral salts-vitamin medium (Table III-5) in the 2 liter shake flask with 20 to 30 mg/ℓ of the test compound. The reservoir for collecting CO_2 contained 10 ml of 0.2 M $Ba(OH)_2$ or 0.5 M KOH (for radiotracer studies). The flasks are sparged with 70 percent oxygen in nitrogen, sealed, and placed on a shaker (125 rpm) in the dark at room temperature. Samples were usually taken at days 3, 7, 14, 21, and 28 with 3 ml of 20 percent H_2SO_4 added the day before termination of the test in order to convert carbonates to CO_2. Duplicate controls were run with inoculum but without test compound and for compounds of unknown stability in water an inhibited ($HgCl_2$) control was run. Glucose, which was used as a readily biodegradable control, typically evolved 80 to 90 percent of the carbon as CO_2 in 28 days and DOC removal was 95 to 100 percent during the same time. Gledhill

Table III-5. Gledhill CO_2 Evolution Medium

	g/ℓ of medium
NH_4Cl	0.035
KNO_3	0.015
$K_2HPO_4 \cdot 3H_2O$	0.075
$Na_2HPO_4 \cdot H_2O$	0.025
KCl	0.010
$MgSO_4$	0.100
$FeSO_4 \cdot 7H_2O$	0.001
$CaCl_2$	0.005
$ZnSO_4 \cdot 7H_2O$	0.0001
$MnCl_2 \cdot 4H_2O$	0.0005
$CuCl_2$	0.00005
$CoCl_2$	0.000001
H_3BO_3	0.000001
MoO_3	0.0000004
One-A-Day multiple vitamin (coat removed)	0.002
Biotin	0.000005
Inositol	0.0001

(1975a) tested a number of radiolabeled and nonradiolabeled LAS products and has also used the procedure with a new detergent builder (Gledhill, 1978) and butyl benzyl phthalate (Gledhill *et al*., 1980).

This CO_2 evolution procedure is in the process of being adopted for standard practice by ASTM (1979a). DOC analysis is encouraged for water soluble compounds and the positive reference compounds that are suggested are sodium citrate, dextrose, phthalic acid, trimellitic acid, and aniline (the use of one or two positive controls is recommended). These controls along with several hydroxyl substituted benzoic acids and mellitic acid have been examined (ASTM, 1979a) and generally gave 80 to 100 percent of theoretical CO_2 evolution.

j. Blok Repetitive Die-Away Test (Blok, 1979)

Blok (1979) in his Repetitive Die-Away Test has attempted to combine the best features of several existing methods. He suggests that his method combines features of the Sapromat method (see Howard *et al*., 1975), the Japanese MITI method, the Fischer Closed Bottle Test, the OECD Modified (for DOC) Screening Test, and the French AFNOR Test. His method has two new elements:

1. "The simultaneous measurement of oxygen uptake and residual amount of organic carbon."
2. "The repetitive incubation."

The mixed culture inoculum for the test is prepared by mixing 70 ml tap water, 10 ml phosphate buffer, 10 ml fresh activated sludge, 10 ml fresh river sediment, 1 ml fresh domestic sewage, and 500 mg fresh compost or garden soil. Following homogenization (ultrasonic mixer for 10 to 20 seconds), the mixture is paper filtered and the filtrate is used as soon as possible (within one hour) by adding 3 ml of the inoculum to each 500 ml of mineral salts medium (see Table III-6).

The test is carried out in triplicate in ground glass-stoppered, brown incubation bottles (280 ml) with teflon magnetic stirrers. The

Table III-6. Blok Repetitive Die-Away Test Mineral Medium (Blok, 1979)

	g/ℓ of medium
KH_2PO_4	0.00283
K_2HPO_4	0.00725
$Na_2HPO_4.2H_2O$	0.01113
$MgSO_4.7H_2O$	0.0075
$CaCl_2$	0.0092
$FeCl_3.6H_2O$	0.000083
NH_4Cl	0.127
Allylthiourea	0.002
Yeast extract	0.005
H_3BO_4	0.0012
$CuSO_4.5H_2O$	0.0012
KI	0.0005
$MnSO_4$	0.0023
$NaMoO_4$	0.0023
$ZnSO_4.7H_2O$	0.0047

concentration of the test compound used (usually 50 mg C/ℓ) normally results in a theoretical oxygen demand of 100 to 150 mg/ℓ. Both a blank (without test compound but with inoculum), a reference, and a toxicity control (made by combining test compound and reference to see if biodegradation of the reference is inhibited by the presence of the test compound) are run. Sodium acetate is used for the reference measurement and each flask is charged with 187 ml of aqueous mixture, carefully closed, and placed on a rotary shaker for one week.

At the end of one week, oxygen is measured with a calibrated oxygen probe and DOC is measured by combining 2.5 ml from each of the three flasks, centrifuging, adding H_2SO_4 to remove carbonates and then determination by a TOC analyzer. After these measurements, the pH is measured and adjusted and the water is aerated. Stock solutions (1.6 ml) of the test compound and/or reference material are added and the volume of the mixture is adjusted to 187 ml. The procedure is repeated weekly for six weeks except that no new test compound is added the last two weeks. With volatile compounds, the liquid mixture should not be aerated and water-insoluble fluids are added by impregnation on a millipore filter. Blok (1979) has run the test with sodium acetate, triethylenetetramine (poorly degraded), diethylhexylphosphoric acid, and kerosine.

k. Miscellaneous

Wendt et al. (1978) used a basal medium in shaker flasks to study the microbial degradation of glycerol nitrates. The medium consisted of 0.2 g of $MgSO_4.7H_2O$, 1.0 g of KH_2PO_4, 1.0 g of K_2HPO_4, 0.02 g of $CaCl_2$, and 0.05 g of $FeCl_3$ in 1 liter of distilled water. The seed for the test run was obtained by inoculating nutrient broth with fresh activated sludge, incubating at 30°C for 18 hours, centrifuging and washing three times in sterile buffer solution and resting for 12 hours at 4°C in sterile phosphate buffer solution. The amount of inoculum placed in the shake flasks was not indicated.

2. Mineral Salts Media - High Microbial Concentration

a. Zahn-Wellens Test (OECD, 1979e; Zahn and Wellens, 1974; Gerike and Fischer, 1979)

The Zahn-Wellens test is meant to represent an industrial sewage treatment plant and is used for evaluating the biodegradability of water soluble, non-volatile organic compounds. The test basically consists of the incubation of activated sludge, mineral nutrients, and test material (50-400 mg/ℓ) (sole carbon source) in a 1-4 liter glass vessel equipped for agitation and aeration. The mixture is agitated or aerated at 22 ± 3°C protected from light for up to 28 days (OECD, 1979e) (before modification by OECD it was 14 day - comparison tests run by Gerike and Fischer (1979) were for 14 days). Samples are taken periodically and the DOC or COD values of the filtered solution determine the ratio of the values at different times to the starting values. This ratio is used to calculate the percentage of biodegradation. The inoculum is activated sludge (0.2-1.0 g/ℓ dry matter in the final mixture) or is taken from a mixture of sources (e.g., different treatment plants, soil extracts, river waters). This inoculum is washed repeatedly by centrifuging or settling with tap water (<5 mg C/ℓ). No preacclimation of the inoculum to the test chemical is used. The lower concentration of inoculum (0.2 g/ℓ) and test material (50 mg/ℓ) were introduced into the method (OECD, 1979e) to make it compatible with the Swiss EMPA Test. At least one control blank is set up to run in parallel with each test series and contains only activated sludge and mineral nutrients.

b. Pitter COD Reduction Test (Pitter, 1976)

Pitter (1976) used a COD reduction test to examine the biodegradabilities of 94 aromatics, 15 hydroaromatics, and 14 aliphatic compounds. Each test compound (approximately 200 mg/ℓ COD) was added to a slightly modified BOD dilution water to which such an amount of thickened adapted activated sludge is added to make the dry weight of the inoculum 100 mg/ℓ. These tests are run in beakers in a dark room (20 ± 3°C) with magnetic stirring. The reduction in the dissolved COD is determined with periodic samples which are filtered or centrifuged before analysis. The following steps are used to develop the thickened acclimated inoculum during a 20 day period: activated sludge aerated in 1000 ml volumetric cylinder; each day 200 ml driven off, sedimentation, 600 ml decanted, residual (200 ml thickened activated sludge) diluted with tap water and 600 mg/ℓ starch or glucose, 600 mg/ℓ peptone, 25 ml phosphate buffer and the solution of the test compound (gradually increasing concentrations), cylinder made up to 1000 ml and aerated 23 hours. This acclimation procedure is very similar to the SDA-SCAS laboratory procedure. Pitter and Fuka (1979) also used this general procedure to study the ultimate biodegradability of linear alkylbenzene sulphonates (LAS); they measured DOC and UV changes as well as COD reduction.

c. Radiorespirometry Biodegradation Tests (Belly and Goodhue, 1976; Belly *et al*., 1975)

Investigators at Eastman Kodak Company have used a radiorespirometric technique to study biodegradation of industrial chemicals by activated sludges in simulated complex industrial effluents (Belly and Goodhue, 1976). In the tests run by Belly and Goodhue (1976) one or two ml of sludge were placed in a serum bottle (30 ml) with 3 ml of mineral salt solution or simulated effluent and 0.2 ml of 1 M phosphate buffer (pH 7.3). The serum bottles were sealed with rubber stoppers and 0.1 or 0.2 ml of radioactive test substrate (27,000-40,000 dpm) were injected into the bottle. Incubation was carried out at room temperature in the dark. In order to collect $^{14}CO_2$, the reaction was stopped with 2 ml of 1 N perchloric acid (kills bacterial cells and acidifies to release $^{14}CO_2$). The released $^{14}CO_2$ is trapped by inserting two cannulas (20 gauge needle) into the serum bottle; one cannula is attached to an air source and the other to two scintillation vials used for subsequent counting. The test is usually run for 5 days (Belly and Goodhue, 1976) although intermediate values are sometimes also measured (Belly _et_ _al_., 1975). Belly and Goodhue (1976) used this procedure to determine the biodegradability of several pure compounds with and without simulated effluent. The procedure has also been used to determine degradation of ethylenediaminetetraacetic acid (EDTA) by a mixed population of microorganisms from an aerated lagoon (Belly _et_ _al_., 1975).

d. Warburg Respirometry

Hart and Helfgott (1975) introduced the term bio-refractory index in which Warburg respirometry was used. The Refractory Index is defined as the Ultimate Biochemical Oxygen Demand (30 day Warburg respirometer corrected for nitrification and raw sewage blank) divided by the Ultimate Oxygen Demand (amount of oxygen needed for complete wet phase oxidation - measured by total nitrogen analysis and total oxygen demand). A number of organic compounds, including natural products, were evaluated with this procedure.

e. Miscellaneous

Urushigawa and Yonezawa (1977) used an activated sludge static test to study the degradation of 31 azo compounds. The azo compounds were added at a concentration of 50 mg/ℓ in 200 ml Erlenmeyer flasks which contained 1 g/ℓ of sludge in a culture medium containing mineral salts and 500 ppm each of glucose and peptone. This mixture was aerated with a rotary shaker at 25 ± 1°C and the disappearance of azo compound was determined in terms of UV absorbance.

3. Natural Aqueous Systems

As indicated in our earlier review, the use of natural aqueous samples as test media for studying the degradation of environmental contaminants has been extensively applied. The general procedure usually consists of

(1) obtaining the environmental sample(s) (can include river, pond, lake, and/or marine water and/or sediments, biological organisms, etc.), (2) treatment and/or stabilization of the sample (e.g., filter suspended sediments out of river water; acclimate water and hydrosoil to an aquarium for a microcosm study), (3) dose the system with the chemical, and (4) follow the disappearance of the parent compound or formation of products. The Proposed Guidelines for Registering Pesticides in the U.S. are the only regulations that recommend these types of tests (see Table III-1).

a. River Die-Away Test

The river die-away test has been used extensively since 1955 to study the biodegradation of chemicals (Howard et al., 1975). The use of surface water for determining the aerobic aquatic metabolism of pesticides is recommended by the U.S. EPA (1978a). The test should be run with radiolabeled chemical and the studies continued until decline curves have been established or for 30 days, whichever occurs first (U.S. EPA, 1978a). Krzeminski et al. (1975) and Schaefer and Dupras (1976) have been referenced by U.S. EPA (1978a) as guidelines for acceptable protocols. Krzeminski et al. (1975) treated 1 liter Schuylkill River water samples with two ^{14}C-isothiazole compounds at three concentrations (1.0, 0.1, and 0.01 ppm). The samples were held in 1 liter glass filtering flasks. Air was bubbled through a tube below the surface of the water and the side arm was used as an outlet tube to collect the $^{14}CO_2$ in a trap. Trap solutions were sampled approximately weekly. Schaefer and Dupras (1976) used a 1-m^2 outdoor pond containing 70 gal of water to determine the decomposition of Dimilin[1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea]. The treated amount provided a calculated concentration of Dimilin (technical grade) of 0.1 ppm and sampling (upper 2 inches of water) was at 1 hour, and 1, 2, 3, and 4 days following treatment. Since the media in the Schaefer and Dupras (1976) procedure contains sediment and vegetation it is probably more similar to a large microcosm study (see Section III.3.c).

A variety of natural water samples and test chemicals have been used with the river die-away test. Various studies representative of the river die-away test, along with details of the investigations, are listed in Table III-7, and there are probably many studies that have not been listed.

b. Water-Hydrosoil Microcosms (Aerobic)

The U.S. EPA (1978a) Guidelines for Registering Pesticides reference a number of articles that use acceptable procedures for determining anaerobic aquatic metabolism with water-hydrosoil systems. The general guidelines indicate that the sediment or soil should be flooded with water and incubated for a month to achieve anaerobic conditions and then the chemical should be added followed by system incubation for up to a year. Those procedures probably result in anaerobic conditions, since Gowda and Sethunathan (1976) found that the redox potential of some flooded soils reached negative values within 2 or 3 weeks. However, there are conditions under which water-hydrosoil systems probably remain aerobic. For example, Miyazaki et al. (1975) [cited by U.S. EPA, 1978a] added 2,6-dichlorobenzamide (0.5 ppm) to a 100 ml suspension of water and hydrosoil (20 g hydrosoil and 80 ml pond water) that was incubated for 40 days at 25°C during which $^{14}CO_2$ evolution was measured in a closed system through which CO_2-free air was circulated. In a similar study, Sikka and Rice (1973) [also cited by U.S. EPA (1978a)] used a 10 gal aquarium containing 7 gal of pond water and 1 1/2 inch layer of pond hydrosoil. After seven days equilibration, the aquaria were treated with endothall (2 and 4 ppm) and samples of water and hydrosoil taken periodically up to 35 days. The redox potential was not measured and the bottom of the tank may not have been aerobic.

Table III-7. River Die-Away Test

Reference	Water Sample	Test Chemical	Analytical Method	Concentration of Test Chemical	Duration of Test
Boethling and Alexander (1979)	Stream (bacterial count 5 x 10^4/ml)	2,4-D, Sevin, p-chlorobenzoic acid	$^{14}CO_2$	Varied to see affect	4-18 days
Saeger and Tucker (1976)	Mississippi River (settled for 2 days)	Phthalate esters	GC-FI	1 mg/ℓ	5 weeks
El-Dib and Aly (1976)	River water	Phenylamide pesticides	TLC	10-100 mg/ℓ (repetitive dosing)	70-140 days
Appleton et al. (1978)	Lake water	3,3'-Dichlorobenzidine	HPLC	--	1 month
Sherrill and Sayler (1980)	Reservoir water	Phenanthrene	GC-FI	0.1-1.0 ppm	4 weeks
Yordy and Alexander (1980)	Lake water	N-nitrosodiethanolamine	$^{14}CO_2$	1 ppm	60 days
Hollis et al. (1976)	Lake water (bacterial count 20-25 x 10^3/ml)	Linear alkyl benzene-sulfonate	Colorimetric (MBAS)	0.05 ppm	28 days
Borighem and Vereecken (1978)	Lake water	Phenol	Colorimetric $^{14}CO_2$	1-20 ppm	300 hours
Reskallah et al. (1979)	Irrigation, drainage, and Nile River water	Leptophos	GC-FI	1 ppm	16 weeks
Sudhakar-Barik and Sethunathan (1978)	Rice field, canal, pond, and river water	Parathion	Colorimetric	1 ppm (repetitive dosing)	7 days
Ward and Brock (1976)	Lake water	Hexadecane, mineral oil	$^{14}CO_2$	10 ppm	100 hours
Drevenkar et al. (1975)	River water	Organophosphorus pesticides	TLC and GC	1-10 ppm	2 weeks
Watson (1977)	River water	2,4-D	UV	50 ppm	180 days
Seesman et al. (1976)	Seawater	Oil	GLC Bacterial growth	0.1%	7 weeks
Lee and Ryan (1976)	Estuarine river water	Hydrocarbons	$^{14}CO_2$	6-30 ppb	96 hours
Atlas (1975)	Seawater	Crude oil	Gravimetric and GLC	100 ppm	42 days
Ward and Brock (1978)	Salt evaporation ponds and Great Salt Lake, Utah	Mineral oil, hexadecane, and glutamate	Dissolved oxygen and $^{14}CO_2$	45-600 ppm	600 hours

Simsiman and Chesters (1976) have studied the degradation of diquat in both aerobic and anaerobic sediment-water systems. These systems were prepared by adding 5 ml fresh sediment and 180 ml water to an Erlenmeyer flask. Aerobic or anaerobic conditions were maintained by passing a stream of oxygen or nitrogen, respectively, into the flask.

Boyle _et_ _al_. (1980) also studied degradation of a pesticide (pentachlorophenol) under aerobic and anaerobic conditions in an attempt to simulate four benthic environments: "(1) the oxygen rich euphotic epilimnion, (2) oxygen rich euphotic littoral zone, (3) low oxygen, dark hypolimnion, and (4) benthic hydrosoil-hypolimnion." This was accomplished by using 50 ℓ aquaria to which were added (1) pond water (illuminated with two 40-W fluorescent lights), (2) pond water and homogenized, autoclaved hydrosoil [illuminated as in (1)], (3) pond water (no illumination and glass top was sealed with silicone rubber sealant), and (4) pond water and homogenized, autoclaved hydrosoil (no illumina-tion and glass top was sealed with silicone rubber sealant). After 45 days the aquaria were treated with 2 mg/ℓ pentachlorophenol.

Gardner _et_ _al_. (1979) also used a water-sediment aerobic system to study the degradation of polycyclic aromatic hydrocarbons in coastal sediments. Hydrocarbons (^{14}C-labeled) were added to sediment-seawater (1 g sediment:50 ml seawater) and incubated in sealed 125 ml bottles with stirring.

Bourquin _et_ _al_. (1977, 1979) and Wilkes (1978) have suggested a core-chamber method for studying the environmental fate of radiolabeled toxicants. In the core-chamber method (Bourquin _et_ _al_., 1977), water and sediment (sand) were collected from a salt marsh, sieved (2 mm^2), and placed in a 10 gal aquaria. The apparatus is depicted in Figure III-5. The water and sediment are acclimated for 24 hours and then the glass cylinders are inserted over the silicone stoppers. Bourquin _et_ _al_. (1979) later modified the procedure

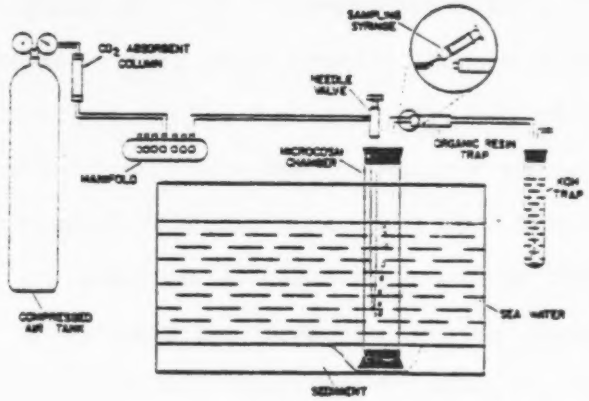
SAMPLING SYRINGE
CO$_2$ ABSORBENT COLUMN
NEEDLE VALVE
MANIFOLD
MICROCOSM CHAMBER
ORGANIC RESIN TRAP
KOH TRAP
SEA WATER
COMPRESSED AIR TANK
SEDIMENT

Figure III-5. Apparatus for core-chamber system (Bourquin *et al.*, 1977).

to include the extraction of intact sediment and water cores from the environment (referred to as Eco-Core). No sieving of the sediment or acclimation is necessary with the use of intact sediment cores. Sterile cores or cores containing no sediment may be examined for comparison.

Eggert _et_ _al_. (1979) used a lake water-hydrosoil system to study the degradation of linear alkylbenzene sulfonates (LAS). They added 3 liters of lake sediment and 8 liters of lake water to a 20 liter aquarium. The aquaria were maintained for 8 weeks with gentle aeration under illumination at 300 foot-candles using GE Daybrite fluorescent lights (16/8 hour light/dark cycle), which allowed stratification of the sediment into aerobic and anaerobic zones and growth of aquatic macrophytes. The redox potentials were not measured. Gledhill _et_ _al_. (1980) used a similar procedure in which 4 liters of lake sediment and 12 liters of lake water were added to a 5 gal aquaria and stabilized as above except only for 6 weeks. The bottom of the aquarium was covered with a 1/8-inch Teflon sheet fitted with eight evenly spaced silicone stoppers. Multiple samples were run in the same aquaria by using a core-chamber technique that was created by inserting eight sterile glass cylinders through the water sediment and over the stoppers. Chambers contained 110 ml of lake water and 35 cm^3 of sediment and were set up to collect $^{14}CO_2$. Samples were taken periodically up to 28 days. This procedure was used to evaluate the fate of butyl benzyl phthalate.

Bourquin _et_ _al_. (1979) have also suggested the use of an Environmental Fate Screening System which consists of 125 ml Erlenmeyer flasks containing water and sediment. Air or nitrogen was passed into the stoppered flask and volatile compounds were collected on XAD-4 resin. Radiolabeled compounds were used and analysis was determined separately on water, suspendable particulate, and non-suspendable sediment or sand (large particles).

c. Aquatic-Terrestrial Microcosm

More complex microcosms which include biota continue to be used by many investigators to determine the environmental transport and fate of pesticides and toxic chemicals. Draggan (1976) has indicated that the following are some of the reasons for the use of microcosms rather than field tests:

1. Afford control of environmental complexity and environmental conditions over a wide range.
2. Amenable to replication at relatively low cost.
3. Circumvent large-scale environmental contamination.

However, it must be kept in mind that microcosms (model ecosystems) are also used for purposes other than to determine the degradation of a chemical. Many focus more on transport (including bioaccumulation) and toxicity.

The two types of model ecosystems [(1) closed model aquatic ecosystem; (2) terrestrial aquatic model ecosystem] developed by Metcalf and his coworkers (see Howard et al., 1975) continue to be used extensively. The details of these studies are tabulated in Table III-8. In addition to the studies listed in Table III-8, a number of investigators have used slightly modified versions of the terrestrial-aquatic model ecosystem.

Tomizawa and Kazano (1975, 1979) used the procedure of Metcalf et al. (1971) procedure except that they substituted the organisms in Figure III-6 because the organisms used in the original method were not available or were difficult to obtain in Japan. Virtanen et al. (1979, 1980) used a slightly modified Metcalf terrestrial-aquatic model ecosystem (organisms used and timing of treatment and sampling were different) with DDT to examine the variability and reproducibility of the model and to study the fate of 4-chloro-2-methylphenoxyacetic acid (MCPA). Caballa et al. (1979) also used the Metcalf terrestrial-aquatic model ecosystem except that the terrestrial portion was unsterilized sandy loam soil instead of white quartz sand. They used the

Table III-8. Recent Studies Using Metcalf Model Ecosystems

Reference	Model Ecosystem	Test Compounds
Lu and Metcalf (1975)	Aquatic	Aniline, anisole, benzoic acid, chlorobenzene, nitrobenzene, phthalic anhydride, hexachlorobenzene, pentachlorophenol, 2,6-diethylaniline, and 3,5,6-trichloro-2-pyridinol
Lu *et al*. (1977)	Aquatic and Terrestrial-aquatic	Benzo[a]pyrene, benzidine, vinyl chloride
Booth (1975)	Terrestrial-aquatic	DDT, Carbofuran, Orthene
Lu *et al*. (1978)	Terrestrial-aquatic	Anthracene, fluorene, carbazole, dibenzofuran, dibenzothiophene
Coats *et al*. (1979)	Terrestrial-aquatic	Nine analogs of DDT

Sweet potato → Tobacco cutworm

Insect larvae excreta → Algae

Red snail

Daphnia → Mosquite larvae

Guppy

Figure III-6. Organisms used by Tomizawa and Kazano (1975, 1979) in the Metcalf Terrestrial-Aquatic Model Ecosystem.

modified procedure to study the environmental fate of drugs and drug metabolites in animal excreta (excreta containing radiolabeled chemicals that had been fed to a turkey were applied to the soil). Metcalf and coworkers (Lee et al., 1976) have also modified the terrestrial-aquatic model ecosystem to simulate a rice paddy in order to study the environmental fate of three dichlorophenyl nitrophenyl ether herbicides. In the rice paddy model ecosystem, white quartz sand was again used to form a soil/air/water interface and 60 4-day-old riceseedlings were transplanted in three rows and four columns to cover the shelf area. The rest of the procedure is the same as used by Metcalf et al. (1971).

An earlier modification of the Metcalf model ecosystem which was developed by Isensee and coworkers (see Howard et al., 1975) at the U.S. Department of Agriculture continues to be used. In that modification the NH_4NO_3 and K_2HPO_4 concentrations were increased 5-fold for satisfactory algae growth, the terrestrial portion was eliminated and the test chemical was adsorbed on soil (representative of the manner in which water insoluble pesticides enter aquatic systems) before the water was added to the system. Isensee (1976) studied the variability of the bioaccumulation potential of several pesticides using this modified procedure. Further modifications have also been incorporated which used (1) a recirculation static system (Isensee et al., 1976), (2) a recirculating static system with compartmental design to allow simultaneous exposure of different trophic level organisms (Kanazawa et al., 1975; Isensee et al., 1979), and (3) a continuous dosing, flow-through system (Isensee and Yockim, 1980). Isensee and Yockim (1980) indicated that the "static system favored pesticide degradation while the flowing system insured continuous pesticide exposure to the organisms." The compartmental design of Kanazawa et al. (1975) has also been used to examine the environmental fate of 2,6-di-tert-butyl-4-methylphenol (BHT) (Inui et al., 1979).

Bourquin et al. (1979) also used continuous flow microcosms. These were used in studies of the fate of methyl parathion both with and without a sediment-water interface. Both large (40 liters) and small (500 ml) reactor vessels were used.

Other model ecosystems have also recently been reported. Schoor (1979) and Tagatz et al. (1975) used an estuarine ecosystem to study the fate of several chemicals. In these studies, 180-liter glass aquaria were used with 160 liters of artificial seawater and a 4-cm layer of beach sand in which 75 turtle grass plants were planted. Seventy-five adult grass shrimp were added and the system adjusted for one week before the chemical was added.

Lichtenstein et al. (1978) have developed a compartmentalized microcosm that consists of terrestrial and aquatic components which can be held separately under a variety of environmental conditions. The apparatus consisted of two stainless steel soil compartments which fed into two glass aquaria. Figure III-7 outlines the sequential steps used with the microcosm.

4. Anaerobic Media

Anaerobic biodegradation tests have been recommended in the EPA Pesticide Guidelines for Registration (U.S. EPA, 1978a) and in the Guidelines for TSCA Premanufacturing Notices (U.S. EPA, 1979); in addition, an anaerobic biodegradability test is being reviewed by ASTM (see Table III-1). These anaerobic biodegradation tests can be divided into two types: (1) mineral salts media-activated sludge digestor seed and (2) flooded soil or sediment.

a. Mineral Salts -- Activated Sludge Digestor Seed

As guidelines for Premanufacturing Notice under TSCA, the U.S. EPA (1979) has recommended a "Methane and Carbon Dioxide Production in Anaerobic Digestion" test which is based upon a method used by Nottingham and Hungate (1969). Nottingham and Hungate (1969) used butyl rubber-stoppered test

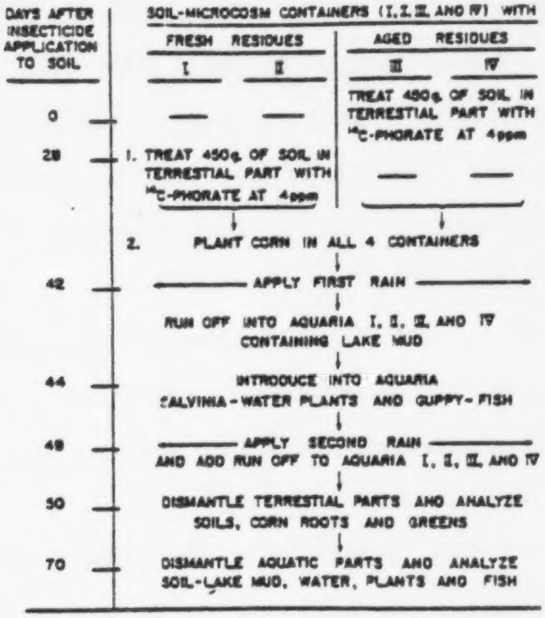
DAYS AFTER INSECTICIDE APPLICATION TO SOIL
SOIL-MICROCOSM CONTAINERS (I, II, III, AND IV) WITH
FRESH RESIDUES
AGED RESIDUES
I
II
III
IV
0
28
42
44
48
50
70
TREAT 450g. OF SOIL IN TERRESTIAL PART WITH 14C-PHORATE AT 4ppm
1. TREAT 450g. OF SOIL IN TERRESTIAL PART WITH 14C-PHORATE AT 4ppm
2. PLANT CORN IN ALL 4 CONTAINERS
APPLY FIRST RAIN
RUN OFF INTO AQUARIA I, II, III, AND IV CONTAINING LAKE MUD
INTRODUCE INTO AQUARIA SALVINIA - WATER PLANTS AND GUPPY - FISH
APPLY SECOND RAIN AND ADD RUN OFF TO AQUARIA I, II, III, AND IV
DISMANTLE TERRESTIAL PARTS AND ANALYZE SOILS, CORN ROOTS AND GREENS
DISMANTLE AQUATIC PARTS AND ANALYZE SOIL-LAKE MUD, WATER, PLANTS AND FISH

Figure III-7. Flow sheet depicting experiments conducted with compartmentalized terrestrial-aquatic microcosm (Lichtenstein _et al_., 1978).

tubes (16 x 150-mm) in which air was displaced with O_2-free CO_2 gas. To the test tube was added 0.5 ml of sludge (from a 5 ml small volume enrichment obtained from a 1 liter anaerobic methanogenic laboratory digestor maintained for several weeks on nutrient broth plus yeast extract and glucose) and 0.5 ml of NYG medium (1% nutrient broth-Difco, 1% yeast extract-Difco, and 1% glucose made up in mineral medium [20 ml of 2.28% $(NH_4)_2HPO_4$ and 20 ml of 3.73% KCl, 4.635% NH_4Cl, 5.075% $MgCl_2.6H_2O$, 1.334% $FeCl_3.6H_2O$, and 0.0783% $CoCl_2.6H_2O$]). The gas volume produced was determined by inserting a needle attached to a 10 ml syringe through the stopper and noting the volume of gas forced into the syringe. The amount of methane and carbon dioxide in the syringe was measured with a vapor refractometer and radioactive $^{14}CO_2$ (from radiolabeled benzoate) was measured by liquid scintillation. The U.S. EPA (1979)-recommended procedure was basically the same with incubation at 35-37°C, daily measurement of gas volume for at least 28 days or until it becomes relatively constant, and the use of twelve paired units containing 0-200 mg/ℓ of test compound.

ASTM (1979b) is in the process of developing a standard practice for determining the anaerobic biodegradability of organic chemicals. They indicated that the Nottingham and Hungate (1969) method had the following drawbacks: "(1) prolonged acclimation period, (2) daily manipulations, (3) lack of indicator to assure maintenance of anaerobic conditions, and (4) relatively imprecise methods for measurement of gas production." ASTM (1979b) decided to use instead a method being used by Healy and Young (1978, 1979; Owen _et al_., 1979). Healy and Young (1978, 1979) used a chemically defined anaerobic medium (see Table III-9) [Healy and Young (1979) also added a 1% (vol/vol) vitamin solution] containing resazurin as an oxidation/reduction indicator. The medium was buffered to pH 7.0 with a bicarbonate-CO_2 system (atmosphere of 30% CO_2 and 70% H_2) and oxygen was removed by boiling and addition of a sulfide reducing

Table III-9. Medium for Anaerobic Biodegradation Test (ASTM, 1979b)

Stock Solutions	Composition	Conc., g/ℓ	Amt. Added/Liter
S-1	Resazurin	0.5	2 ml
S-2	$(NH_4)_2HPO_4$	20	
	NH_4Cl	100	2 ml
S-3	$CaCl_2$	18	
	$MgCl_2 \cdot 6H_2O$	180	
	KCl	130	
	$MnCl_2 \cdot 4H_2O$	2	
	$CoCl_2 \cdot 6H_2O$	3	10 ml
	H_3BO_3	0.6	
	$CuCl_2$	0.23	
	MoO_3	0.6	
	$ZnCl_2$	0.2	
S-4	$FeCl_2 \cdot 4H_2O$	368	1 ml
S-5	$NaHCO_3$	-	2.64 g
S-6	$Na_2S \cdot 9H_2O$		10 ml

agent. To the medium was added 10% (v/v) anaerobic digestor supernatant from a local domestic waste water treatment plant. The tests were run in 250 ml serum bottles into which the 300 mg/ℓ of the test substrate had been added. The procedure for anaerobic transfer of defined media into the serum bottles is illustrated in Figure III-8. The bottles were incubated at 35°C in the dark and the gas produced was measured with a syringe (20 ml) and needle (20 gauge) as in the Nottingham and Hungate (1969) procedure. Both the Healy and Young (1978, 1979) investigations studied aromatic substrates, the disappearance of which was also measured by diluting a centrifuged sample and analyzing it with a spectrophotometer. Acclimated stock cultures were obtained by removal of one-fifth of the culture volume and replacement with fresh, prereduced media. This medium could then be used as inoculum for another bottle.

ASTM (1979b), in its proposed standard practice, has incorporated some slight variations. For example, they recommend 125 ml serum bottles, 50 mg/ℓ carbon of test substrate, and the gas production is measured using a pressure transducer. Pressure readings should be taken at days 3, 7, 14, 21, and 28. With compounds that have low water solubility, it is recommended that the compound be dissolved in dioxane and a solvent blank be added to the controls. Triplicate serum bottles for each chemical are recommended as is the use of ethanol as a reference compound.

In an attempt to see if there was a correlation between the rate of anaerobic degradation and the redox potential of a chemical, Geer (1978) used an anaerobic test system which consisted of anaerobic sludge (2 ℓ) in a 2 liter flask in an incubator box. The flasks were equipped with a combination reference (Ag/AgCl) and pH (glass) electrode, platinum wire electrode, a nitrogen and sample inlet and a sampling port, and the contents were magnetically stirred. The sludge was collected on Friday and incubated over the weekend with

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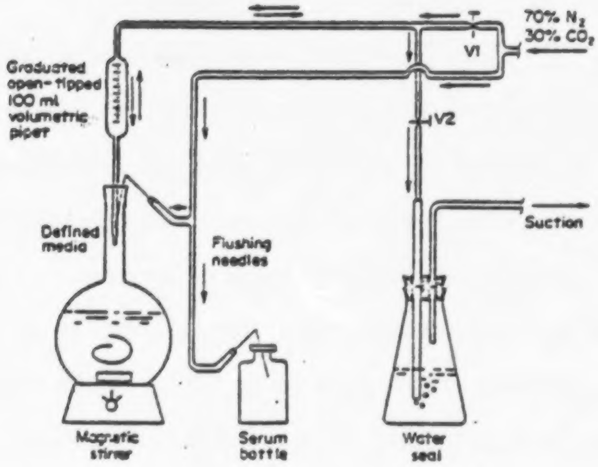
70% N_2
30% CO_2
V1
Graduated open-tipped 100 ml volumetric pipet
V2
Suction
Defined media
Flushing needles
Magnetic stirrer
Serum bottle
Water seal

Figure III-8. Diagram of procedure for anaerobic transfer of defined media into serum bottles (Owen et al., 1979).

8 grams of dehydrated Bacto Nutrient Broth. The redox potential (Ag/AgCl vs. Pt) initially rose above -320 mV but had recovered by Monday (about -480 mV). Usually 20 mg of the test compound (dissolved in 10 ml of acetone) were added and its degradation was followed by GC analysis of aliquots. Although the redox potential of the compounds did not correlate with the rates of their degradation, the redox potential of the sludge was important to the rate of degradation of some compounds.

b. Flooded Soil or Sediment

As previously reviewed under Water-Hydrosoil Microcosms (Aerobic), it is possible that systems containing water and soil or sediment may be aerobic or anaerobic, depending upon the conditions used. The U.S. EPA (1978a) Guidelines for Registering Pesticides suggests the use of flooded soil or sediment as an anaerobic aquatic metabolism test. However, of the references cited (Gowda and Sethunathan, 1976; Hance and Chesters, 1969; Miyazaki et al., 1975; Sikka and Rice, 1973 - see Table III-1), only Gowda and Sethunathan (1976) measured the redox potential of the system. They found that the redox potential of most soils reached negative values (anaerobic) within 2 or 3 weeks, but that one sandy soil and one extremely acidic soil still showed positive values (i.e., still aerobic) after 25 days. Therefore, flooding soil or sediment and incubating for a month, as recommended by U.S. EPA (1978a), does not necessarily mean that anaerobic conditions are achieved. Two of the studies cited by U.S. EPA (1978a) as containing acceptable procedures for conducting anaerobic aquatic metabolism studies (Miyazaki et al., 1975; Sikka and Rice, 1973), used little (7 days) or no equilibration of the sediment-water system and, therefore, were probably aerobic.

Two investigators have used water-sediment systems under aerobic and anaerobic conditions by passing a stream of oxygen or nitrogen, respectively, through the system. Simsiman and Chesters (1976) used an experimental set-up similar to that used by Hance and Chesters (1969) [cited by U.S. EPA, 1978a] that consisted of 5 ml of fresh sediment, 180 ml of water, and 5 ppm herbicide incubated at room temperature in a stream of O_2 or N_2. Bourquin et al. (1979) also used sediment-water in a flask with air or nitrogen introduced as an Environmental Fate Screening System. Boyle et al. (1980) have also suggested that anaerobic conditions were achieved by incubation of water-hydrosoil for 45 days before chemical treatment. None of these investigations measure redox potential or used an oxidation/reduction indicator, such as resazurin, to indicate whether this system was anaerobic.

Ward and Brock (1978) have studied the oxidation of 1-^{14}C-hexadecane in sediment-water or sediment-prereduced mineral media. When sediment-water was used, bottom water clarified septically through a Millipore filter was gassed under N_2 to remove oxygen. For the other system, the prereduced mineral-salts medium was prepared by autoclaving the mineral salts and reducing the system (used methylene blue as an indicator) with a reducing agent and by bubbling N_2 through the system. The tests containing samples of dilute sediment suspension (approximately 2% dry weight) were run in sterile serum vials.

5. Pure Culture and Cell-Free Extract Studies

Pure cultures of microorganisms as well as cell-free extracts continue to be used in studies of the biodegradation of environmental contaminants. These types of studies are particularly useful in isolating metabolic intermediates in order to determine the pathways of degradation. Also, isolation of a microorganism that can metabolize a chemical does provide suggestive evidence that a chemical may biodegrade in nature. The U.S. EPA (1978a) has recommended that these types of studies be undertaken in order to register pesticides. However, the fact that the conditions used (defined mineral media, no other source of carbon) in these types of studies differ greatly from natural conditions (for example, enrichment culture may isolate a microorganism that is unable to degrade the chemical in natural water due to lack of enzyme induction, low population, etc.) makes interpretation of results from cell-free extracts or pure culture studies extremely difficult in terms of environmental significance. A large number of these types of studies have been conducted and a sampling is presented in Table III-10. Most of these studies provide some insight into pathways of degradation and the isolation of large numbers of microorganisms that can degrade chemicals is suggestive of an ability of the chemical to biodegrade in nature. In a few instances, pure culture studies have been used to estimate biodegradation rate constants to provide kinetic values for modeling effects (Wolfe *et al.*, 1978; Smith *et al.*, 1979). The use of pure cultures or other techniques to provide kinetic biodegradation rates will be discussed in the evaluation section.

B. Dynamic Aqueous Biodegradation Techniques

Dynamic (continuous or semi-continuous) biodegradation procedures continue to be used mostly to simulate waste water treatment and also to reproduce environmental conditions (e.g., continuous culture). One advantage of dynamic systems is that toxic products and metabolic wastes will generally accumulate to a lesser extent and their effect on the degradation process will not increase with time. These systems are more like activated sludge treatment plants or the natural aquatic environment where a continual removal or dilution of the toxic products is expected. There are many differences between systems that attempt to simulate the natural aquatic environment and those that attempt to simulate activated sludge treatment plants. For example, the temperature, microbial concentration and types of microorganisms, food source, and acclima-

Table III-10. Pure Culture and Cell-Free Extract Studies

Reference	Test Chemical	Organism Isolated	Source
Sikka and Saxena (1973)	Endothall	*Arthrobacter*	Lake water and hydrosoil
Van Alfen and Kosuge (1974)	2,6-Dichloro-4-nitroaniline	*Escherichia coli* *Pseudomonas cepacia*	Researchers
Bohonos *et al*. (1977)	*p*-Cresol, methyl parathion, benzo[b] thiophene, dibenzothiophene, 9H-carbazole, quinoline, benzo[f]quinoline, benz[a]anthracene, benzo[a]-pyrene, 7-H-dibenzo[c,g]-carbazole, Mirex, 2,4-D	---	Natural waters
Ellis (1977)	Phenol, catechol	6 pure cultures	Commercial sources
Mahaffey *et al*. (1979)	Phenylacetic acid	PAL-1, -10, and -100	Lake water
Mulkins-Phillips and Stewart (1974)	Fuel oil	*Nocardia* sp.	Beach sand or seawater
Mackrell and Walker (1978)	Quaternary ammonium compounds	*Pseudomonas* sp.	Soil
Gibson (1977)	Xylenes, dibenzothiophene, PCBs	*Pseudomonas* sp. *Nocardia* sp. *Beijerinckia* sp.	Not stated
Cox and Goldsmith (1979)	Ethylbenzene, 1-phenethanol	*Nocardia tartaricans*	Commercial sources
Jigami *et al*. (1979)	*n*-Propylbenzene	*Pseudomonas* sp.	Not stated
Griffin and Cooney (1979)	Pristane, 1,13-tetradecadiene, cyclohexane, and benzene	35 pure cultures	Water and sediment

Table III-10. Pure Culture and Cell-Free Extract Studies (Cont'd)

Reference	Test Chemical	Organism Isolated	Source
McKenna and Heath (1976)	Polynuclear aromatic hydrocarbons	*Pseudomonas putida* *Flavobacterium* sp. Cell-free extracts	Soil and water
Walker *et al*. (1976)	Petroleum	*Vibrio* sp. *Pseudomonas* sp. *Acinetobacter* sp.	Sediment
Jamison *et al*. (1976)	High octane gasoline	32 organisms	Groundwater
Gibson (1975, 1976)	Benzo[a]pyrene Benz[a]anthracene	Mutant strain of *Beijerinckia*	Not stated
Watkinson and Somerville (1976)	Butadiene	*Nocardia* sp.	Water and soil
Donoghue *et al*. (1976)	Cyclohexane	*Nocardia* sp. *Acinetobacter* sp. Cell extracts	Soil
Schaefer *et al*. (1979)	Octanes	27 organisms	Researchers
Fall *et al*. (1979)	2,6-Dimethyl-2-octene	*Pseudomonas citronellolis*	Researchers and commercial sources
Cundell and Traxler (1973)	Petroleum	Six types of bacterium	Sediments
Bourquin (1977)	Malathion	15 organisms, mostly pseudomonad type	Salt-marsh
Walker and Colwell (1976)	Long-chain *n*-alkanes	Five axenic cultures	Not stated

Table III-10. Pure Culture and Cell-Free Extract Studies (Cont'd)

Reference	Test Chemical	Organism Isolated	Source
Hou *et al*. (1979a)	Secondary alcohols	Methane- and methanol-utilizing microbes	Lake and soil
Hou *et al*. (1979b)	C_2 to C_4 *n*-alkenes	20 new methane-utilizing microbes	Lake and soil
Rosenberg and Alexander (1979)	Organophosphorus pesticides	*Pseudomonas* sp.	Soil and sewage
Daughton and Hsieh (1977)	Parathion	*Pseudomonas stutzeri*	Sewage and agricultural runoff
Blakley (1978)	Cyclohexanecarboxylic acid	*Achromobacter* sp.	Soil
Furukawa and Matsumura (1976)	PCBs	*Alkaligenes* sp.	Lake sediment by enrichment using biphenyl
McCormick *et al*. (1976)	2,4,6-Trinitrotoluene	*Veillonella alkalescens* cell-free extract	Not stated
Dorn and Knackmuss (1978)	Substituted benzoates and catechols	*Pseudomonas* sp.	Commercial source
Furukawa *et al*. (1978)	PCBs	*Alkaligenes* sp. *Acinetobacter* sp.	Sediment
Bourquin and Przybyszewski (1977)	Nitrilotriacetate	*Pseudomonas* sp.	Freshwater and estuarine water
Maeda *et al*. (1978)	Nicotine-N'-oxide	*Arthrobacter globiformis*	Tobacco leaves and soil

Table III-10. Pure Culture and Cell-Free Extract Studies (Cont'd)

Reference	Test Chemical	Organism Isolated	Source
Rzechowska (1976)	Surfactants (ethylene oxide)	72 strains of bacteria	Activated
Hartmann *et al*. (1979)	3-Chloro-, 4-chloro-, and 3,5-dichlorobenzoate	*Pseudomonas* sp.	Not stated
Knackmuss and Hellwig (1978)	Chlorinated phenols	*Pseudomonas* sp.	Sewage
Reineke and Knackmuss (1979)	Chlorinated benzoates	Novel strain of *Pseudomonas* sp. (plasmid transfer)	---
Matsumura and Benezet (1973)	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	100 Microbial strains	Previously isolated
Watson and Jones (1975)	Polyethylene glycols	*Acinetobacter* SC25 *Pseudomonas* KW8 *Flavobacterium* BT1	Sewage
Smith and Callely (1975)	Cyclohexanecarbocylic acid	*Arthrobacter* sp.	Marsh mud
Ripin *et al*. (1975)	Alkylbenzenes sulfonates	*Pseudomonas testosteroni*	Previously isolated
Blakley (1974)	Cyclohexanecarboxylic acid	*Arthrobacter* sp.	Garden soil
Lewis *et al*. (1975)	Malathion	Fungus, *Aspergillus oryzae*	Freshwater pond
Geller (1980)	Atrazine	Enriched from ten sites - not identified	Soils, waters, activated sludge
Yagi and Sudo (1980)	PCBs	Alcaligenes BM-2	Soil
Bauer *et al*. (1979)	2,4-D	*Pseudomonas* sp.	Garden soil

tion vary considerably. It should also be emphasized that in terms of environmental risk the above two processes that can be simulated by dynamic systems are quite different: one (simulated activated sludge) is a wastewater treatment process which may prevent release of contaminants to the environment; the other attempts to simulate how a chemical will behave once it reaches the environment. The results from one system are not necessarily applicable to the other.

1. Continuous Flow

a. OECD Confirmatory Test

The official German test method for detergents that was reviewed in our previous report has become known as the OECD Confirmatory Test. This method as well as some slight modifications has been used by a number of investigators (Howard et al., 1975). To review briefly, the apparatus used, depicted in Figure III-9, consists of a complete mixing aerator (3 ℓ) and settler (2.2 ℓ) with an air pump sludge return. A synthetic sewage containing nutrients and 20 ppm of surfactants is fed at a rate of 1 ℓ/hour. The air flow into the aerator provides agitation and is adjusted to maintain a dissolved oxygen level at above 2 ppm. The sludge is developed as a result of sewage seed or chance inoculation from the air and is allowed to build up to a concentration of about 2500 mg/ℓ. It is presumed that the sludge is acclimated because it is developed in the presence of the test chemical. The effluent from the apparatus is collected in 24-hour composite samples and analyzed for methylene blue active substances (MBAS) when anionic compounds are being tested. The percent degradation is calculated by comparing the influent content to the effluent content and taking the average of the daily values from a 21 day period that maintained a reasonably steady removal of detergent. Stennet and Eden (1971) suggested a "porous pot" modification to overcome difficulties with recirculation of the sludge. The basic difference is that the sludge is

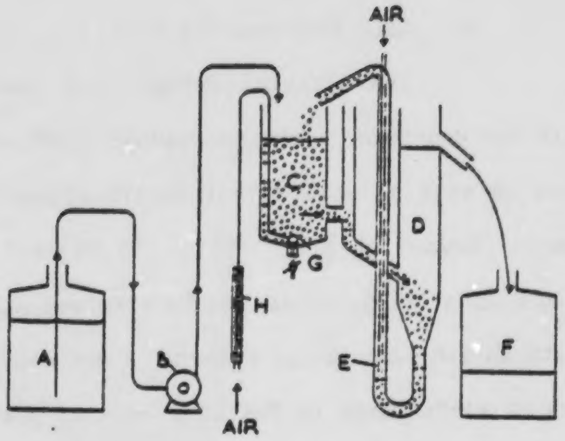

Figure III-9. Apparatus for OECD Confirmatory Test: A, storage vessel; B, metering pump; C, aeration vessel; Cp, porous aeration vessel; D, sedimentation vessel; E, air-lift pump; F, effluent collection vessel; G, sintered-glass diffuser; H, air-flow meter; I, outer impermeable vessel (Stennet and Eden, 1971).

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separated from the effluent by retention in a porous polyethene vessel (Figure III-10) rather than by flocculation and settling in a clarifier.

Painter and King (1978) used the OECD Confirmatory Test and the "porous pot" modification to determine the effect of temperature and phosphate on biodegradation. The configuration of the OECD Confirmatory Test was slightly modified (Figure III-11). The tube delivering returned sludge to the aeration chamber was bent to prevent losses by splashing over the side and an extra diffuser stone was added to prevent suspended solids from settling. Also, the porous pot configuration (Figure III-12) was slightly different from that of Stennett and Eden (1971). The synthetic sewage that Painter and King (1978) used and that is recommended for the OECD Confirmatory Test consisted of 160 mg peptone, 110 mg of beef extract, 30 mg of urea, 7 mg of NaCl, 4 mg of $CaCl_2 \cdot 2H_2O$, and 2 mg of $MgSO_4 \cdot 7H_2O$ dissolved in 1 liter of tap water. They found that occasionally this feed did not contain enough phosphate and, therefore, recommended the addition of 5 mg/ℓ of phosphate in order to prevent phosphorus-deficiency, which results in erratic removal of detergent.

Gerike _et al_. (1978) have used the OECD Confirmatory Test to study the biodegradation of four quaternary ammonium salt surfactants. Some of these compounds had bactericidal properties and required that lower concentrations be used for high removal (>80%) rates.

Fischer and Gerike (Fischer _et al_., 1975; Fischer and Gerike, 1975) have modified the OECD Confirmatory Test in order to use carbon analysis rather than specific chemical analysis; this is referred to as the Coupled Units OECD Confirmatory Test. In principle, the test consists of running two activated sludge units in parallel, one with added test material and one without (Fischer _et al_., 1975). The amount of removal is determined by comparing the unspecific analysis (Chemical Oxygen Demand-COD or Dissolved

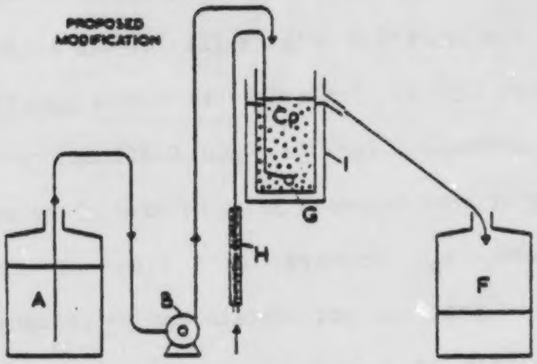

Figure III-10. Apparatus for Porous Pot Modification of the OECD Confirmatory Test: A, storage vessel; B, metering pump; C, aeration vessel; Cp, porous aeration vessel; D, sedimentation vessel; E, air-lift pump; F, effluent collection vessel; G, sintered-glass diffuser; H, air-flow meter; I, outer impermeable vessel (Stennet and Eden, 1971).

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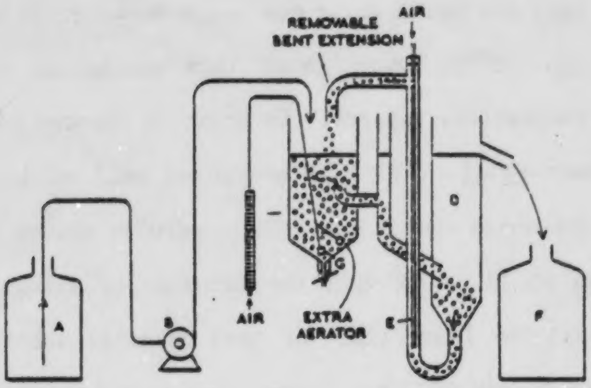
REMOVABLE BENT EXTENSION
AIR
A
B
AIR
C
EXTRA AERATOR
D
E
F

Figure III-11. OECD Confirmatory Test, slightly modified (Painter and King, 1978).

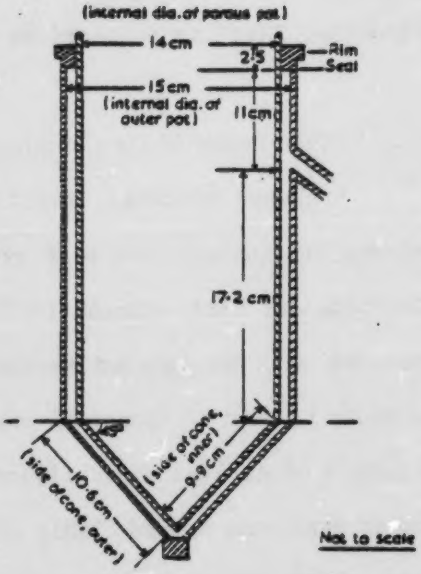
(internal dia. of porous pot)
14cm
2.5
Rim
Seal
15cm
(internal dia. of outer pot)
11cm
17·2 cm
45°
(side of cone, inner)
9·9 cm
10·6 cm
(side of cone, outer)
Not to scale

Figure III-12. Porous Pot (Water Research Centre, Stevenage, England) Modification of OECD Confirmatory Test (Painter and King, 1978).

Organic Carbon-DOC) of the dosed and undosed sludge unit. This requires the assumption that the units would run truly parallel if both were treated equally. Fischer et al. (1975) investigated this assumption with a MBAS detergent and found poor statistical support. In order to improve and assure parity of the two units, Fischer et al. (1975) interchanged half of the activated sludge vessel volumes of the units once a day. Their results showed that the confidence levels can be drawn at 10 or 5% with the exchange of sludge. Fischer et al. (1975) indicated that the lower limit of test chemical concentration should be around 40 ppm COD or 20 ppm DOC, but higher concentrations should be used when possible to narrow the tolerance limits. Fischer and Gerike have applied this method to several detergents of different chemical classes (Fischer and Gerike, 1975) and to 44 compounds covering wide ranges of physical, chemical and biological properties (Gerike and Fischer, 1979; Gerike, 1977). In the latter study, the Coupled Units OECD Confirmatory Test was compared to results for six static biodegradability tests.

b. Other Bench Scale Continuous Flow Activated Sludge Systems

A number of other investigators have elected to use their own activated sludge continuous flow test systems that are somewhat different than the OECD Confirmatory Test. Brink (1976) used polyacrylic plastic units that were constructed with an aerated section (10 cm wide, 16 cm long) and a 1 liter settling basin (return of microbial solids to aerated section by gravity) and contained 3 liters of aerated mixed liquor. Peristaltic pumps were used to feed 3 liters/day of synthetic sewage (Table III-11). By comparing the BOD, COD, TOC, and in some cases the specific chemical analysis of influent to the effluent, it was possible to calculate percent removal. The test was used with a number of biocides (chlorophenols, acrolein, dithiocarbamates, and dibromonitrilopropionamide).

Table III-11. Brink (1976) Synthetic Sewage Feed

A. Salt Solution		B. Concentrated medium	
$MgCl_2 \cdot 6H_2O$	18.6 g	Glucose	15 g
$CaCl_2 \cdot 2H_2O$	36.4 g	Nutrient broth	15 g
$FeCl_3 \cdot 6H_2O$	0.25 g	KH_2PO_4 Sodium Benzoate	15 g 15 g
H_2O	to 1 liter	H_2O	to 500 ml

The concentrated medium is sterilized by autoclaving. Each solution is stored at 4°C until used. The feed consists of 1 ml of A + 10 ml of B + tap water to 1 liter, for each liter of synthetic sewage.

Tabak and Barth (1978) used 5.7 liter capacity units of the type previously used by Ludzack in 1960. The feed was settled (0.5 hr), domestic waste water, the decant of which was passed through two layers of cloth gauze. The feed was maintained in 20 liter polyethylene aspirator bottles in a refrigerator at 5°C and metered into the reactors with diaphragm pumps at a rate of 4 ml/min (hydraulic retention time of 24 hours). The biodegradation of benzidine was studied by monitoring the influent and effluent.

Wendt _et_ _al_. (1978) used an apparatus that consisted of two 2000 ml reagent bottles with tubulations for overflow at 1500 ml, connected in series with a final 500 ml conical sedimentation vessel for solids recovery and return to the first reagent bottle. The contents of the reagent bottles were aerated (3.2 ℓ/min) and magnetically stirred. The medium for the continuous flow activated sludge studies was composed of 2 g of nutrient broth (Difco) per liter of distilled water (buffered to pH 7.0) and the inoculum was 10 ml of aerated activated sludge from a domestic treatment plant. This procedure was used to study the biodegradation of glycerol nitrate.

c. Miniature Continuous Flow Activated Sludge Units

The Swisher _et_ _al_. (1967) miniature (300 ml) continuous flow activated sludge units continue to be used. Gledhill (1978) used such a unit with sieved (74 µ porosity screen), refrigerated natural sewage to study the biodegradation of a detergent builder (carboxymethyltartronate). A pump was used to deliver 50 ml/hr (6 hr detection time) and the mixed liquor suspended solids (MLSS) were maintained at 2500 mg/ℓ. Gledhill (1975b) also used the miniature unit to study the ultimate biodegradation of 3,4,4'-trichlorocarbonilide but used an influent feed rate of 30 ml/hr (10 hr retention time) and maintained 4000 mg/ℓ of MLSS. Both of these studies used a modification of the miniature unit to allow for collection of $^{14}CO_2$ (Figure III-13).

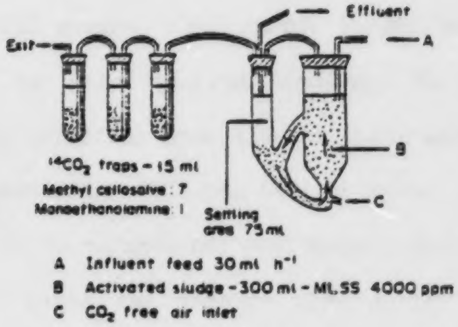
Effluent
Exit
A
B
C
$^{14}CO_2$ traps - 15 ml
Methyl cellosolve : 7
Monoethanolamine : 1
Settling area 75 ml
A Influent feed 30 ml h^{-1}
B Activated sludge - 300 ml - ML.SS 4000 ppm
C CO_2 free air inlet

Figure III-13. Continuous flow activated sludge units for monitoring $^{14}CO_2$ Evolution from ^{14}C-labeled compounds (Gledhill, 1975b).

Kimerle and Swisher (1977) used a modified miniature unit that was similar to the "porous pot" modification of the OECD Confirmatory Test. The 300 ml continuous flow activated sludge unit that they used (Figure III-14) was equipped with a porous poly[illegible]ylene filter candle to separate the siphoning effluent liquid from the sludge solids. The MLSS was maintained between 2500 and 3500 mg/ℓ and a synthetic sewage (OECD Confirmatory Test) was fed to the unit. Effluent from the unit that was fed LAS was used for toxicity tests. Gledhill (1978) also used the porous filter unit to study the biodegradation of carboxymethyltartronate.

Gledhill (1978) developed a laboratory model contact stabilization activated sludge unit (Figure III-15) in order to determine the biodegradation of carboxymethyltartronate at short retention times. The aeration and reaeration vessels were initially filled with acclimated activated sludge at MLSS levels of 1000 and 4000 mg/ℓ, respectively, and natural, sieved, domestic sewage was pumped into the aerator at 100 ml/hr (1.5 hr detention time). The motorized Teflon wall scraper was added to improve solids settling and recycle and an additional feed line was added to increase the feed rate to 150 ml/hr (retention time of 1.0 hr) twice daily (7 am to 12 noon; 6 pm to 11 pm).

d. Continuous-Culture

Continuous-culture chemostats are usually run at microbial concentrations considerably lower than would be found in activated sludge, usually in an attempt to simulate natural aqueous conditions. Pritchard _et_ _al_. (1976) used a three stage (Figure III-16) continuous culture apparatus to study oil biodegradation. In the first vessel, 250 ml of Lake Ontario water amended with 0.7 mM phosphate buffer (pH 7.2), 0.19 mM ammonium chloride, and 0.08 mM magnesium sulfate was placed with eight milliliters of sterile diesel oil. This

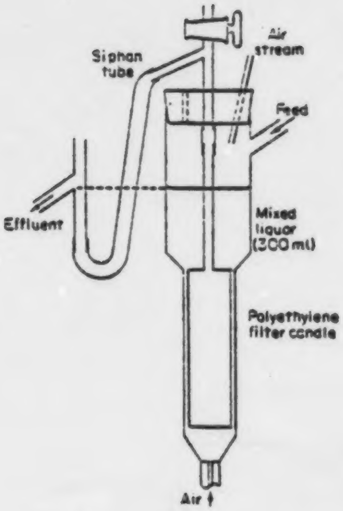
Siphon tube
Air stream
Feed
Effluent
Mixed liquor (300 ml)
Polyethylene filter candle
Air

Figure III-14. Laboratory continuous flow activated sludge unit with polyethylene porous filter candle (Kimerle and Swisher, 1977).

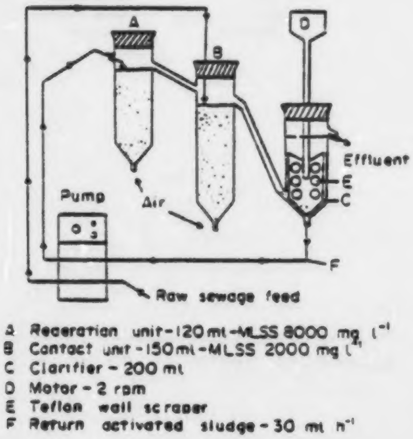
A
B
D
Effluent
E
C
Pump
Air
F
Raw sewage feed
A Reaeration unit-120 ml-MLSS 8000 mg l⁻¹
B Contact unit-150 ml-MLSS 2000 mg l⁻¹
C Clarifier - 200 ml
D Motor - 2 rpm
E Teflon wall scraper
F Return activated sludge - 30 ml h⁻¹

Figure III-15. Laboratory model contact stabilization activated sludge system (Gledhill, 1978).

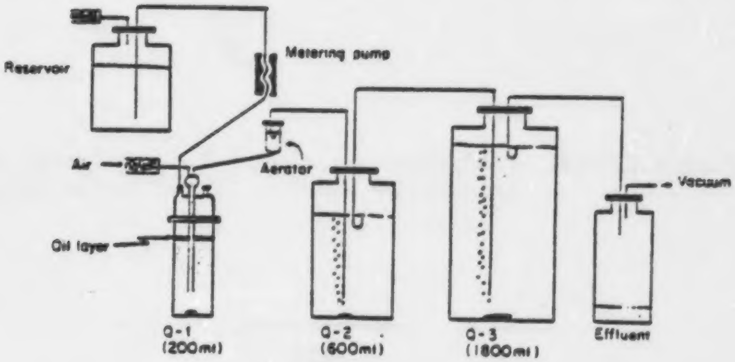
Reservoir
Metering pump
Air
Aerator
Vacuum
Oil layer
Q-1
(200ml)
Q-2
(600ml)
Q-3
(1800ml)
Effluent

Figure III-16. Multistage continuous culture apparatus used to study the microbial degradation of oil (Pritchard et al., 1976).

system was incubated as a batch culture for 12-15 hours and then the flow of reservoir medium was commenced at a dilution rate of 0.05 per hour. Changes in the composition of the oil in the three stages were monitored by gas chromatography.

Wendt et al. (1978) also used continuous-culture experiments to study the biodegradation of glycerol nitrates (GTN) in a medium that simulates the composition of waste water known to result from GTN manufacture (11 mg of K_2HPO_4, 0.7 mg of NaCl, 3.6 mg of $MgSO_4 \cdot 7H_2O$, 0.57 ml of ethanol, 0.07 ml of anhydrous ether, and 2.86 mg of $NH_4H_2PO_4$ per liter of water). The studies were conducted in a chemostat with agitation at 400 rpm, aeration at 0.8 ℓ/min, temperature of 30°C, and detention time of 8 to 15 hours with a working volume of 350 ml. Inocula were provided by adding 10 ml of aerated activated sludge from a domestic sewage treatment plant.

2. Semi-Continuous Flow

a. Soap and Detergent Association Semi-Continuous Activated Sludge (SCAS-SDA)

The semi-continuous activated sludge (SCAS) procedure developed for ABS-type surfactants by the Soap and Detergent Association's (SDA) Subcommittee on Biodegradation (SDA, 1965) continues to be used with a large variety of chemicals. The SCAS-SDA procedure uses a 24-hour cycle consisting of 23 hours for aeration (1.5 liters of sewage and sludge) and 1 hour for settling, drawing off the effluent (1 liter), and filling with fresh feed. The aeration chamber consists of a cylinder with a cone shaped bottom, the dimensions of which are illustrated in Figure III-17. The activated sludge, initially obtained from a sewage treatment plant, is maintained at a mixed liquor suspended solids (MLSS) level of 2500 ± 500 mg/ℓ by discarding solids as necessary. The synthetic sewage that is used consists of 13.0 g glucose, 13.0 g nutrient broth, 13.0 g beef

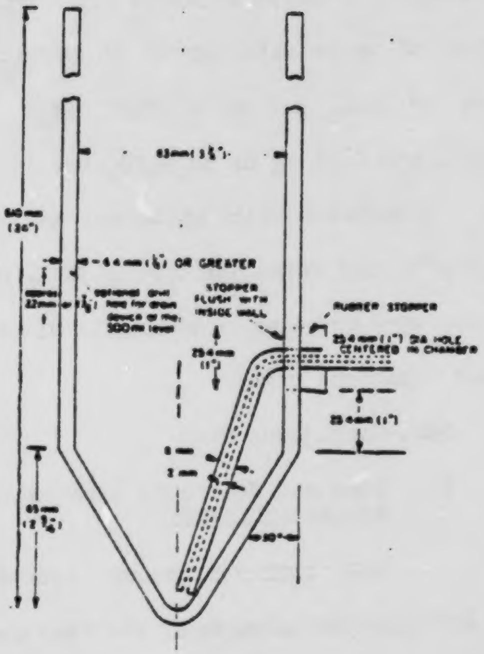
83 mm (3¼")
610 mm (24")
6.4 mm (¼") OR GREATER
22 mm (⅞")
STOPPER FLUSH WITH INSIDE WALL
RUBBER STOPPER
25.4 mm (1") DIA HOLE CENTERED IN CHAMBER
25.4 mm (1")
25.4 mm (1")
8 mm
2 mm
65 mm
30°

Figure III-17. Soap and Detergent Association semi-continuous activated sludge (SCAS-SDA) aeration chamber (SDA, 1965).

extract, 13.0 g dipotassium hydrogen phosphate, 2.5 g ammonium sulphate in one liter of tap water. The minimum operation time is 15 days - 5 for surfactant build-up, 3 for equilibration at 20 mg/ℓ, and 7 for level operation.

The U.S. EPA has recommended the use of the SCAS-SDA procedure for pesticide registration testing (U.S. EPA, 1978a; Krzeminski et al., 1975) and for premanufacturing testing (U.S. EPA, 1979). The pesticide guidelines require the use of radiolabeled chemical and a procedure to allow $^{14}CO_2$ collection from SCAS-SDA has been suggested by Huddleston and Nielson (1979). The reference cited by the U.S. EPA (1978a) as containing an acceptable procedure for pesticide registration (Krzeminski et al., 1975) omitted fill and draw procedures over weekends and instead continued aeration for that period.

Larson (1979) has recommended that the SCAS-SDA procedure be used along with a CO_2 evolution procedure to estimate biodegradation potential. One change in the procedure is that he recommends analysis of effluent samples for DOC instead of methylene blue active substances. However, this change limits the application of the method to water-soluble chemicals.

Several other investigators have used the SCAS-SDA procedure to study the treatability of a number of commercial chemicals including phosphate esters (Saeger et al., 1979), phthalic acid esters (Saeger and Tucker, 1976), adipic acid esters (Saeger et al., 1976), and PCBs (Tucker et al., 1975). These studies all used a modified feed suggested by SDA (1969).

b. Miniature SCAS

Gledhill (1978) used a 300 ml SCAS unit which was fed daily sieved (74 μM) domestic raw sewage with 50 mg/ℓ of the test compound (carboxymethyltartronate). The SDA procedure was followed except that the removal was determined by comparison of influent and effluent DOC values.

C. Analytical Procedures

Both direct (e.g., analysis of parent compound or its metabolite) and indirect (e.g., measurement of oxygen or CO_2 consumption) analytical methods continue to be used to study biodegradation in aqueous systems. However, recent emphasis has been placed on the use of indirect methods especially for application in screening tests. Gerike (1977) has suggested two reasons for using indirect methods in screening tests:

> "1. The development of specific analyses for the great number of compounds to be investigated would be an unsurmountable task.
> 2. Unspecific analyses usually present a better measure of the extent of biodegradation than for specific analyses."

The following indirect (summary parameters - Gerike, 1977) methods have been used:

- BOD (biochemical oxygen demand) in respirometric tests
- CO_2 production, also in respirometric tests
- COD (chemical oxygen demand, usually determined by wet combustion with dichromate) in various tests
- DOC (dissolved organic carbon, usually determined by catalytic combustion after separation of particulates including biomass) in various tests

The first two parameters are primarily measurements of energy metabolism. Gerike (1977) has suggested that they are not quite quantitatively conclusive indicators of biodegradation since they do not measure the sum of energy metabolism and biomass production. COD is widely used but has a number of uncertainties (Gerike, 1977) (some compounds only poorly determined - pyridine and acetic acid; nitrite analyzed by COD - increment not due to organic material). On the other hand, DOC does not have a problem of nitrite interference, but can only be used with water soluble compounds. In addition, DOC requires a certain level of test chemical (5-50 mg DOC/ℓ) in order to be above the background (approximately 5 mg DOC/ℓ) and these concentrations may be toxic to the microorganisms (Blok, 1979; OECD, 1979c). Also, DOC removal gives an all or nothing measurement of biodegradability as is indicated below (Gerike, 1977):

$$CH_3OH \longrightarrow HCOOH \longrightarrow CO_2 + H_2O$$

67% COD removal   100% COD removal

0% DOC removal   100% DOC removal

Thus, the analytical method selected depends upon the physical-chemical properties of the chemical and the information required. A number of investigators have provided direct comparisons of various direct and indirect analytical procedures and these will be described in detail in later parts of this section.

An analytical procedure usually consists of two parts: (1) the extraction and cleanup and (2) the analysis method. Only a few new extraction and cleanup procedures have been used since our last report.

1. Extraction and Cleanup

Shiaris et al. (1980) have evaluated the applicability of a Tenax-GC extraction technique for use in aqueous biodegradation assays with polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). They found the method to be quantitative and reproducible for phenanthrene (a PAH), but variable and not quantitative for PCBs. Also aqueous sample volume and varying concentrations of organic matter influenced PCB and PAH extraction efficiency. Shiaris et al. (1980) conclude that the technique was useful for phenanthrene but not for PCB analysis in biodegradation assessments.

Boyer et al. (1977) (subcommittee of Soap and Detergent Association) developed an analytical procedure for nonionic surfactants that could be used in biodegradation studies. The procedure consisted of a foaming step for concentrating and an ion-exchange step for isolation.

2. Analytical Techniques

Very few new analytical procedures have been developed and applied to aqueous biodegradation tests. Boyer et al. (1977) (SDA subcommittee) have formally recommended the use of cobalt thiocyanate active substances (CTAS) along with foaming isolation (sublation) for biodegradation studies of nonionic surfactants. Fischer et al. (1975) evaluated the use of COD and/or DOC elimination for the OECD Confirmatory Test. They found that the test had to be modified to allow the use of indirect measurements. The test procedure incorporated a step of exchanging half the activated sludge volumes of a control and treated vessel (coupled units) daily in order to increase the probability of both units being equal. Ultraviolet (UV) absorption has been used previously as an indicator of biodegradation. Urushigawa and Yonezawa (1977) have again used UV absorption to study the biodegradation of 31 azo dyes, all of which strongly absorbed UV light.

3. Direct Comparisons of Various Analytical Procedures

Numerous investigators have compared the results obtained on the same test biodegradation system with varying direct and indirect analytical methods. Many studies have used more than one indirect parameter to measure biodegradation. For example, Fischer (1975) measured % $BODT_{30}$ (BOD/COD, 30 days), % DOC loss, and % CO_2 production with the pure oxygen version of the Closed Bottle Test. These results are shown in Table III-12. In general, the various methods gave comparable results. Gledhill (1975a) found comparable values for both percent theoretical CO_2 evolution and % DOC removal (Table III-13) with the Gledhill CO_2 evolution system. Larson (1979) measured both % CO_2 evolution and % DOC removal (Table III-14). Gerike and Fischer (1979) found similar results with CO_2 evolution and DOC removal in the Sturm (1973) test and with $BODT_{14}$ and DOC remaining with the MITI test upon examination of 44 organic chemicals.

Several studies have compared results between direct analysis and indirect analysis with varying results in terms of comparability. For example, Tobin et al. (1975) compared the conventional Wickbold procedure for analyzing

Table III-12. Biodegradability in Closed Bottle Test using Various Analytical Procedures (Fischer, 1975)

	Closed Bottle Test/air saturated Version	Closed Bottle Test/pure oxygen Version		
	% $BODT_{30}$	% $BODT_{30}$	% DOC-loss	% CO_2 production
Na-n-Dodecylsulfate (Control)	92	87	80	81
Na-Citrate (Control)	85-92	100	100	121**)
Propane 1,1,2,3-tetracarboxylic acid	77	77	88	86
Ethane-1,1,2,2-tetracarboxylic acid	41	32	28	32
O-CM-hydroxy-succinic acid	53	53	88	87
O-CM-tartaric acid	17	24	7	11
Bis-O-CM-tartaric	0	10	0	0
Polyacrylic acid	7	--	--	--
Polyhydroxyacrylic acid	14	7	23*)	0
Polyoxycarboxylic acid 1	51	49	38	39
Polyoxycarboxylic acid 2	16	20	18	24
Maleic acid-propylene cooligomer	0	--	--	--
Cyclopentane tetracarboxylic acid	0	0	0	2

all acids as sodium salts

*) possible loss by ultrafiltration

**) CO_2 from inoculated cell material included

Table III-13. Ultimate Biodegradation of LAS (Gledhill, 1975a)

Type	Alkyl chain length	% Theoretical CO_2 evolution	Dissolved organic Carbon removal (%)
Commercial LAS products	$C_{10.8\ avg}$ to $C_{11.7\ avg}$	64	75
	$C_{12.8\ avg}$	47	68
	$C_{13.3\ avg}$	37	59
Pure LAS homologues	C_9 to C_{12}	77	85
	C_{13}	61	63
	$C_{14,15}$	49	65
	$C_{13,14}$[a]	69	84
	C_{15}[a]	58	76

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[a] Substrates were added to the test medium incrementally - on days 0 and 1,5 mg/liter; on days 3 and 7, 10 mg/liter.

Table III-14. Biodegradation of Soluble Surfactants (Reprinted from Larson, 1979. Estimation of biodegradation potential of xenobiotic organic chemicals. Appl. Environ. Microbiol. 38(6):1153-1161, with permission from the American Society for Microbiology.)

		Observed	
Compound	Length of expt (days)	Total CO_2 (%)	DOC removed[a] (%)
LAS I-10	30	72.9	81
LAS I-20		67.3	
LAS II-10	30	48.4	55
LAS II-20		37.0	
Glucose-20		86.0	100
AE I-10	29	95.0	100
AE I-20		99.8	
AE II-10	24	98.7	100
AE II-20		86.5	
Glucose-20		96.5	100
Zwit I-10	42	41.0	49
Zwit I-20		--[b]	--
Zwit II-10	33	19.7	25
Zwit II-20		17.4	
Glucose-20		97.7	100
CAP I-10	35	32.3[c]	31
CAP I-20		26.8	
CAP II-10	35	0.0	0
CAP II-20		0.0	
Glucose-20		92.0	100

[a] Average removal.

[b] Insufficient data for determination of constants.

[c] Percent TOC converted to CO_2.

polyethoxylate nonionic surfactants to a hydrogen bromide ether cleavage, gas chromatographic method. They found that although the conventional procedure indicates complete degradation early in the test (OECD Screening Test), the gas chromatographic method shows that only the alkyl portions of the molecule are degrading and the polyethoxylate degrades only slowly or incompletely. In contrast, Huddleston and Nielson (1979) found that for linear alkylsulfonates in a SCAS system the percent loss measured colorimetrically (MBAS) was the same as the percent of ring loss measured by $^{14}CO_2$ evolution (^{14}C randomly ring labeled).

Saeger and Tucker (1976) showed that the time frame for results from various analytical procedures may differ considerably. For example, with butyl benzyl phthalate (Figure III-18) biodegradation in a SCAS system was fairly complete in 6 hours as measured by GC of the parent compound, but intermediate products still remained after 12 hours as measured by ultraviolet spectrophotometry of the water soluble intermediates.

Healy and Young (1978) studied the degradation of phenol under anaerobic conditions by comparing the UV absorption to CH_4 and Co_2 evolution (Figure III-19). They also found time frame differences that were attributable to the different analytical methods.

In studying the biodegradation of EDTA using radiorespirometry, Belly _et al_. (1975) compared both a GLC technique and a total nonvolatile carbon (TOC) technique to the radioisotope method, which they felt could underestimate degradation. During a 5 day period, the percent degradation was 28-30%, 89%, and 63% as measured by $^{14}CO_2$ evolution, GLC, and TOC, respectively.

D. Evaluation of Techniques Used for Determining Biodegradation in Water

Biodegradation test methods can be evaluated in a number of different ways including comparing the results obtained from one test to the results from another and, wherever possible, comparing field experiments and/or ambient

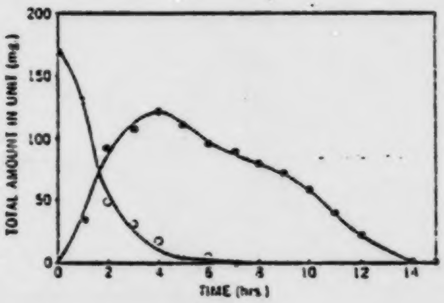

Figure III-18. Butyl benzyl phthalate in SCAS. ○ BBP, ● water soluble intermediates (Saeger and Tucker, 1976).

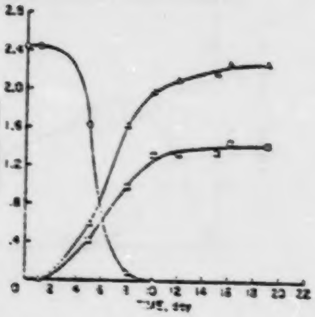

Figure III-19. Phenol carbon balance under anaerobic conditions. Symbols: ○ millimoles of phenol carbon; △ cumulative millimoles of total gas produced; □ cumulative millimoles of methane produced (Healy and Young, 1978).

monitoring data to laboratory results. Several comprehensive comparisons of various test methods have recently been carried out (e.g., Gerike and Fischer, 1979) and these will be reviewed in detail in the following sections. However, little new information is available that would allow comparisons of field and laboratory results. Some significant work has been undertaken in an attempt to determine the quantitative rates of biodegradation and the progress in that area will be evaluated in detail in one of the following sections.

Quantitative results from different degradation test methods and from field studies have varied considerably, and perhaps this explains why assignment of quantitative rates of biodegradation has not even been attempted. The factors which affect biodegradation are numerous and have varied from test to test and from laboratory test to natural conditions. Therefore, it is useful to discuss these factors to understand how and to what extent they are responsible for discrepancies in the degradation results.

1. Factors Affecting Biodegradation

a. Type of Inoculum

The source and extent of acclimation of the inoculum can have a considerable affect on the results of a biodegradation test. However, this is not always the case. For example, Kondo and Jujii (1978) studied the degradation of sorbic acid, m-chloroaniline and aniline using a river die-away test procedure with river water from two sources and seawater from two sources. The degradation curves for all the water samples were remarkedly similar. In contrast, Borighem and Vereecken (1978) found that non-polluted river water had a significant lag time in the river die-away degradation of phenol, while phenol was degraded rapidly in river water that was exposed to steelwork effluent. Similarly, Watson (1977) found that the rate of 2,4-D degradation on river water was significantly affected by the season (see Figure III-20). The rate of

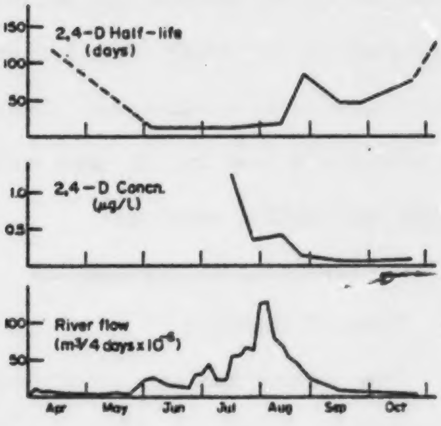

Figure III-20. Variation in half-life and natural concentration of 2,4-D in Avon River water (Watson, 1977).

degradation was demonstrated to be related to the concentration of 2,4-D in the river (possible acclimation) and river flow (Figure III-20). Similar seasonal results were noted by Yordy and Alexander (1980) (Figure III-21).

Acclimation of the inoculum either in nature (contaminated water or sludge) or in the laboratory prior to use in a test has been shown to have considerable impact on the biodegradation results (Howard _et_ _al_., 1975). Acclimation of the microorganisms can be unpredictable and acclimation to a chemical under one set of conditions does not quarantee degradation under another set of conditions. For example, Hollis _et_ _al_. (1976) compared the degradation of linear alkyl benzenesulfonate (LAS) with acclimated and unacclimated seed and found some significant differences in the rate of degradation. Using a miniature continuous flow activated sludge (CFAS) unit, Gledhill (1978) demonstrated that acclimation to carboxymethyltartronate was slowly lost but was regained rapidly upon reintroduction into the CFAS unit.

Healy and Young (1978) indicated that the source of inoculum may also be important in anaerobic test systems. In most cases, they had to wait 4-5 weeks before any evidence of degradation appeared. However, in one case in which a different inoculum was used, 7 weeks were necessary before catechol degradation began.

The microbial inoculum used in some of the screening biodegradation test systems has varied considerably. Both activated sludge and soil have been used as well as natural waters; some procedures recommend an acclimation step while others do not (this can be particularly important in procedures that are run for a specific period of time). The different microbial inoculum for these test systems are summarized in Table III-15.

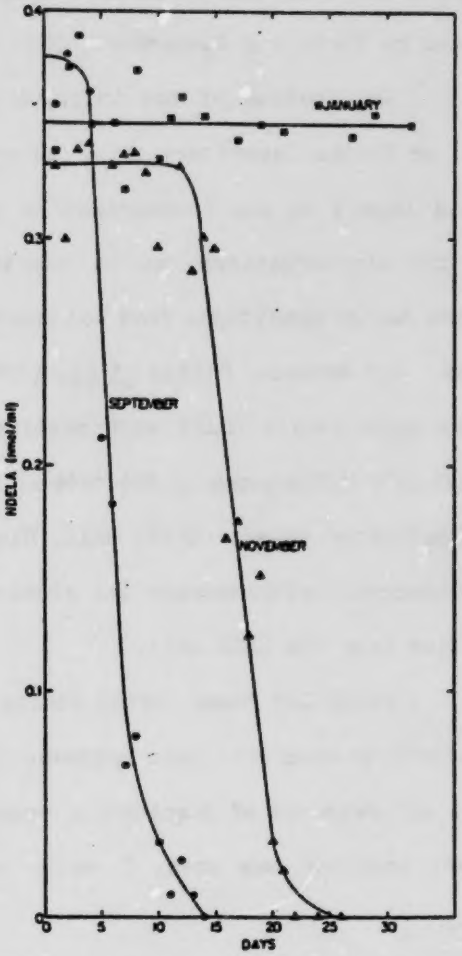

Figure III-21. **Effect of season on N-nitrosodiethanolamine metabolism in lake lake water (Reprinted from Yordy and Alexander, 1980. Microbial metabolism of N-nitrosodiethanolamine in lake water and sewage. Appl. Environ. Microbiol., 39(3):559-565, with permission from the American Society for Microbiology.)**

Table III-15. Microbial Inoculum for a Variety of Screening Tests

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BOD Test - Filtered effluent from a biological treatment plant (0.1-0.5 ml/liter of mineral salts medium

Japanese MITI Test - 1ℓ sludge, 1ℓ surface water, 1ℓ surface soil obtained four times a year at not less than ten places; stirred in container, filtered, aerated 30 minutes, 1/3 supernatant removed and equal volume of 0.1% synthetic sewage added; repeated each day; final medium 30 ppm (w/v)

Closed Bottle Test - Soil (100 g/liter tap water) or activated sludge effluent, filter, discard first 200 ml, inoculum 1 drop/liter - should not exceed 10^3 to 10^5 germs/ml

Sturm CO_2 Evolution Test - Acclimated culture of 150 ml settled influent sewage, 1350 ml BOD water, 50 mg/liter yeast extract, 20 mg/liter test chemical; incubate for 14 days; filter; inoculate 1:10

Larson CO_2 Evolution Test - Acclimated effluent from SDA-SCAS procedure (20 mg/liter test chemical), homogenize, settle 15-30 minutes, inoculate (1:99 = 10^4 to 10^5 CFU/ml in final medium)

SDA Presumptive Test - Mixed culture (1 ml transfer to 100 ml of fresh medium every week) inoculum; two 72 hr adaptive transfers with test chemical (1 ml/100 ml)

OECD Screening Test - Unacclimated inoculum from secondary effluent (domestic treat-ment plant) (0.5 ml/liter)

French AFNOR Test - Three samples from surface water (must contain 10^5 bacteria/ml), microbes membrane-filtered and added to mineral salts solution ($5 \pm 3 \times 10^7$ germs/ml measured by adsorbance at 620 nm)

Gledhill Shake-Flask CO_2 Evolution Test - Acclimated inoculm prepared from 2.5 g/liter topsoil and 50 ml/liter freshly settled domestic sewage in mineral salts and 25 mg/liter each of Difco vitamin-free Casamino Acids and yeast extract; mixed 10-15 minutes, screened, incubated 14 days with test chemical, add 5 vol. inoculum to 1 vol. settled raw sewage = final inoculum; 60 ml inoculum to 440 ml mineral salts medium

Blok Repetitive Die-Away Test - Mix 70 ml tap water, 10 ml phosphate buffer, 10 ml fresh activated sludge, 10 ml fresh river sludge, 1 ml fresh domestic sewage, and 500 mg fresh compost or garden soil; homogenize, filter, add 3 ml inoculum to 500 ml mineral salts media

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b. Mineral Salts Composition and Supplementary Nutrients

For proper growth and function of microorganisms in biodegradation tests, elements such as nitrogen, phosphorus, sulfur and magnesium at relatively high concentrations, and various other elements such as iron, copper, manganese and zinc in trace amounts, are required. The type of medium used can determine whether the microorganisms are in one of two phases: (1) growing cells (degradation is linked to growth of microorganisms) and (2) resting cells (degradation is a result of preformed enzymes). Resting cells are generally suspended in a buffer alone whereas growing cells have a complete medium.

As is apparent from the data presented in earlier sections, there are almost as many different mineral salts media reported as there are degradability studies. This is illustrated in Table III-16 for a number of the screening biodegradation tests. The exact effect on the biodegradation results of these different medium has not been determined although, in some cases, rationales have been given for modifications. For example, Gerike and Fischer (1979) modified the OECD Screening Test by tripling the concentration of ammonium so that an optimal C:N:P ratio was obtained. The unmodified OECD Screening Test used standard BOD dilution water (see Table III-16). Gerike and Fischer (1979) also added trace metals and an essential vitamin solution to the OECD Screening Test medium in order to optimize test conditions. Whether this increase in ammonia, trace metals, or essential vitamins has any effect is unknown.

Ward and Brock (1976) examined environmental factors that affected hydrocarbon oxidation in a river die-away test (used lake water). During summer, they found that nutrient deficiencies limited hydrocarbon biodegradation. Addition of nitrogen and phosphorus resulted in higher rates of degradation.

Table III-16. Mineral Salts Media for Various Aqueous Biodegradation Test Protocols (mg/ℓ of medium)

	Standard BOD Water*	SDA Presumptive Test	Larson CO_2 Evolution Test	French AFNOR Test	Gledhill CO_2 Evolution Test	Blok Repetitive Die-Away Test
KH_2PO_4	8.5		17	300		2.83
K_2HPO_4	21.75	1000	43.5		75	7.25
$Na_2HPO_4 \cdot 7H_2O$	33.4		66.8	2000	25	11.13
NH_4Cl	1.7	3000			35	127
$(NH_4)_2SO_4$				300		
NH_4NO_3				150		
$MgSO_4 \cdot 7H_2O$	22.5	250		50	100	7.5
$CaCl_2$	27.5			50	5	9.2
KI						0.5
KCl		250		10		
KNO_3				15		
$FeCl_2 \cdot 6H_2O$	.025		0.1			.083
$FeSO_4 \cdot 7H_2O$		2		10	1	
$MnSO_4$				10		2.3
$MnCl_2 \cdot 4H_2O$					0.5	

Table III-16. Mineral Salts Media for Various Aqueous Biodegradation Test Protocols (mg/ℓ of medium) (continued)

	Standard BOD Water*	SDA Presumptive Test	Larson CO_2 Evolution Test	French AFNOR Test	Gledhill CO_2 Evolution Test	Blok Repetitive Die-Away Test
$NaMoO_4$						2.3
K_2MoO_4				2.5		
MoO_3					0.0004	
$Na_2B_4O_7 \cdot 10H_2O$				2.5		
H_3BO_3					0.001	1.2
$Co(NO_3)_2 \cdot 6H_2O$				2.5		
$CoCl_2$					0.001	
$CuCl_2 \cdot 2H_2O$				2.5	0.05	
$CuSO_4 \cdot 5H_2O$						1.2
$ZnCl_2$				2.5		
$ZnSO_4 \cdot 7H_2O$					0.1	4.7
NH_4VO_3				1.0		
One A Day Multiple Vitamins					2	
Biotin					0.005	
Yeast Extract		300		5		5
Inositol					0.1	

*The following tests also used standard BOD water - Sturm, OECD Screening Test.

In another study, Ward and Brock (1978) examined the biodegradability of mineral oil, hexadecane, and glutamate in natural samples of varying salinity and found that the rates of metabolism decreased as salinity increased. Their results suggest that biodegradation of hydrocarbons in hypersaline media is doubtful.

A number of other investigators have demonstrated that other supplemental nutrients, especially carbon, can have considerable impact on the rates of biodegradation in a test system. For example, Belly et al. (1975) found that the addition of several amino acids or sugars strongly inhibited biodegradation of the ferric chelate of ethylenediaminetetraacetic acid (EDTA). Belly and Goodhue (1976) have compared the $^{14}CO_2$ evolution from a number of chemicals with and without simulated photographic processing effluent (Table III-17). They found that for some compounds (hydroquinone and formaldehyde) the rate of degradation decreased while with others (phenol and benzoic acid) a marked stimulation in degradation was noted.

Painter and King (1978) have demonstrated that the phosphorus content of synthetic sewages used in continuous activated sludge biodegradation tests can have a considerable effect on sludge growth and test chemical removal. Thus, they recommended the addition of 5 mg P/l to the OECD Confirmatory Test to ensure that phosphorus-deficiency does not occur.

c. Test Chemical Concentration

Boethling and Alexander (1979a,b) have studied the effect of test chemical concentration on biodegradation using a number of model compounds including p-chlorobenzoate, chloroacetate, 2,4-D, Sevin, 1-naphthol, glucose, dimethylamine, diethylamine, and diethanolamine. They measured the rate of $^{14}CO_2$ evolution of the compounds at various concentrations (ppt - ppm range) in

Table III-17. Radiorespirometric Studies Using ^{14}C-Chemicals in the Presence and Absence of an Industrial Effluent

Chemical	% $^{14}CO_2$ recovery without effluent 5 days	% $^{14}CO_2$ recovery in presence of effluent 5 days	% Degradation based on BOD_5 values[a]
Benzoic acid-1-^{14}C	68.2	86.9	80.2–83.8
Benzyl alcohol-7-^{14}C	85.7	88.9	60.8
Formaldehyde-U-^{14}C	18.2	10.1	3.4–66.1
Glycerol-U-^{14}C	75.2	N.D.[b]	43.5–52.9
Hydroquinone-2,3,5,6^{14}C	65.2	46.3	25.4
Phenol-1-^{14}C	18.1	46.5	22.7–88.2
Sodium acetate-U-^{14}C	93.2	90.8	34.6–85.9
Sodium cyanide-^{14}C	25.7	N.D.	N.D.
Sodium ferrocyanide-^{14}C	1.7	N.D.	0.3

[a] See Heukelekian and Rand, 1965.

[b] Not determined by these workers.

stream water. With the chemicals studied they were able to divide them into two groups: (1) a group in which the rate is directly proportional to the initial concentration (10 or 100-fold lower concentration would result in 10 or 100-fold lower rate) (amines, p-chlorobenzoate, chloroacetate) and (2) a group in which little or no degradation may occur at low concentrations, and a threshold below which no significant mineralization occurs (2,4-D, Sevin, and 1-naphthol). Results for 2,4-D are illustrated in Figure III-22. The relationship between concentration and rate is predicted by Michaelis-Menten kinetics for enzyme reactions (Boethling and Alexander, 1979a) but a threshold is not. Boethling and Alexander (1979a) were not surprised to find a threshold and suggested that one explanation is that the energy obtained from metabolism of the small amount of substrate is too low to meet the energy demands of the initially small population that is active on the compound. These results have significant impact on extrapolations from laboratory tests to the prediction of biodegradation in natural waters since most tests are run at concentrations well above concentrations that are likely to be found in nature.

In a similar study, Borighem and Vereecken (1978) examined the degradation of phenol in river water at different initial concentrations (Figure III-23). Although the range of concentrations was not as great as used by Boethling and Alexander (1979a,b), a slight difference in rate, especially initially for higher concentrations, is noticeable.

In evaluating the Closed Bottle Test, Gerike and Fischer (1979) have indicated that the method gives high evaluations when biodegradation is strongly concentration dependent (e.g., diethylene glycol, hydroquinone, and LAS).

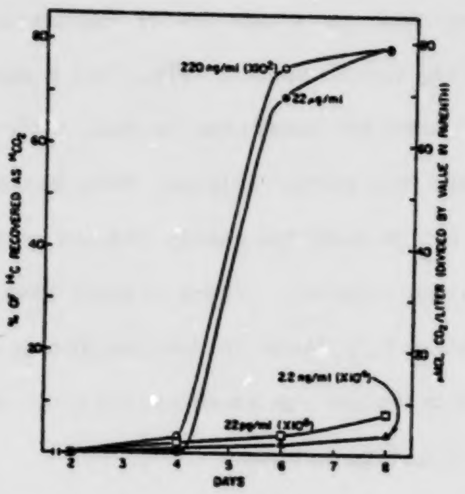

Figure III-22. Foundation of Co_2 from 2,4-D added to stream water at four initial concentrations (Reprinted from Boethling and Alexander, 1979. Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. Appl. Environ. Microbiol., 37(6):1211-1216, with permission of the American Society for Microbiology.)

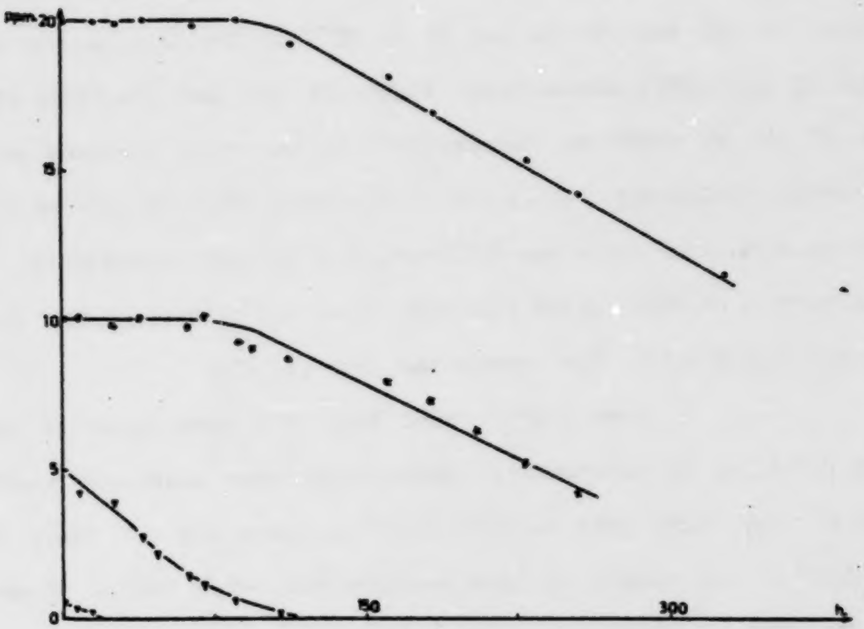

Figure III-23. Degradation of phenol in stirred water from the River Samber (Borighem and Vereecken, 1978).

d. Temperature, pH, Light, etc.

A number of studies have demonstrated that temperature is an important factor in biodegradability tests. Belly et al. (1975) indicated that the optimum degradation of NH_4Fe-EDTA occurred at room temperature (22°C); at 3°C there was a 49% decrease. Ward and Brock (1976) determined that the temperature optimum for oil degradation was 20 to 25°C in the lake waters they studied. Hollis et al. (1976) demonstrated (Figure III-24) that the ratio of biodegradation of LAS at different temperatures in the river die-away procedure were considerably different. Atlas (1975) determined that the rate of degradation of heavy oils vs. light oils was different at different temperatures. At 20°C, the toxic portion of the lighter oils evaporated and allowed greater biodegradation than with heavy oils. The reverse was true at 10°C.

Geer (1978) found that with some types of compounds the redox potential is important to degradation under anaerobic conditions. For example, they found that tetrachloronitrobenzene did not react if the redox potential of the sludge was less negative than -0.38 volts. However, overall with the chemicals they studied, they did not find a correlation between degradation and redox potential.

Borighem and Vereecken (1978) investigated whether light had an effect on the degradation rate of phenol in river water. They found no detectable effect.

2. Comparison of Biodegradation Methods

The most extensive comparison of various test methods was conducted by Gerike and Fischer (1979). They examined 44 chemicals using seven different biodegradation methods: (1) OECD Coupled Units; (2) Zahn-Wellens, (3) MITI, (4) AFNOR, (5) Sturm, (6) OECD Screening, and (7) Closed Bottle Test. The

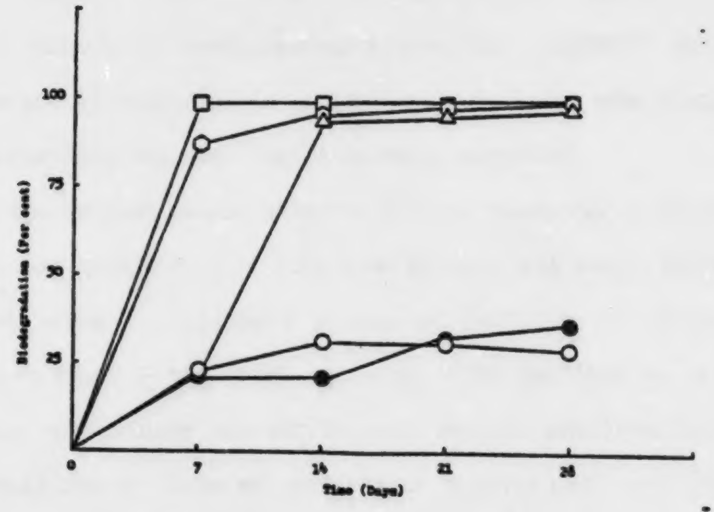

Figure III-24. Biodegradation of LAS at different temperatures using an unacclimated seed in the river die-away procedure; ○, 5°C; △, 15°C; ⬡, 25°C; □, 35°C; ●, 45°C (Hollis *et al.*, 1976).

results of these comparisons are tabulated in Table III-18 and organized into groups of chemicals in which the results from the tests were (1) Full Agreement--Negative--7 chemicals, (2) Full Agreement--Positive--12 chemicals, (3) Low Results Only in Closed Bottle and/or MITI--9 chemicals, (4) Discrepancies Among Other Results--10 chemicals, and (5) Inhibitory Materials--6 chemicals. The last four compounds in Table III-18 are tetrapropylenebenzenesulfonate (TBS), linear alkylbenzenesulfonate (LAS), benzyldimethyldodecylammonium chloride (BDMDAC), and cetyltrimethylammonium bromide (CTMAB). Not all the chemicals were examined in the tests, as indicated by the dashes in Table III-18.

The seven compounds that were non-biodegradable in all tests were generally in agreement with literature values (Gerike and Fischer, 1979). In the situation where low results were only obtained with the Closed Bottle Test, the procedure was modified by adding essential vitamins and trace metals (same amounts as modified OECD Screening Test) and a Sturm preacclimation procedure. These alterations changed five of the six results for chemicals that were previously low, thus perhaps suggesting the need for modification of the procedure. The results for the 10 chemicals that gave varying results for different tests could be partially explained. For example, the positive result for _t_-butanol in the Zahn-Wellens and OECD Screening Tests could be partially due to stripping or evaporation; the DOC loss for _t_-butanol in the Sturm test obviously was due to stripping since it was not accompanied by CO_2 evolution. The Sturm test gave varying results from one run to another for 2- and 3-chlorobenzoic acid, sulfanilic acid and 3-aminophenol, thus questioning its reproducibility. 3-Chlorobenzoic acid was only degraded in the Zahn-Wellens, Sturm, and the square wave feeding version of the OECD Coupled Units Test. Gerike and Fischer (1979) indicate that acclimation appears to be important for this compound; however, the Zahn-Wellens procedure does not incorporate an acclimation step. The inhibitory

Table III-18. Biodegradability in Various Tests (Reprinted from Gerike and Fischer, 1979. A correlation study of biodegradability determinations with various chemicals in various tests. Ecotoxicol. Environ. Saf., 3(2):159-173, with permission of Academic Press, Inc., New York.)

Compound No.	Compound	Coupled units test	Zahn-Wellens test		MITI test		AFNOR test	Sturm test		OECD Screen. test	Closed bottle test
		DOC rem. ± Tol$_{95\%}$ (%)	DOC rem. (%)	After days	DOC rem. (%)	BODT$_{14}$ (%)	DOC rem. (%)	CO_2 evol. (%)	DOC rem. (%)	DOC (%)	BODT$_{30}$ (%)
FULL AGREEMENT IN ALL TESTS: RESULTS NEGATIVE											
1	Cyclopentanetetra-carboxylic acid	4 ± 5^a	0	14	0	16	--	0	19	8	0
2	Polyvinyl alcohol (PVA)	-1 ± 2	11	14	0	12	--	0	0	--	0
3	Carboxymethyl-cellulose (CMC)	23 ± 3	16	14	--	--	--	8	7	--	25
4	Ethylenediamine-tetraacetic acid (EDTA)	-5 ± 4	37	14	--	--	0	10	22	10	3
5	Ethylene oxide-propylene oxide block polymer	-2 ± 4^a	19	14	8	2	38	0	0	11	4
6	1,3-Benzene-disulfonic acid	--			7	15	--	0	0	11	2
7	3-Aminobenzene-sulfonic acid	-8 ± 6	0	14	2	6	--	0	5	0	0

Table III-18. Biodegradability in Various Tests (Gerike and Fischer, 1979)

Compound No.	Compound	Coupled units test DOC rem. ± Tol$_{95\%}$ (%)	Zahn-Wellens test		MITI test		AFNOR test DOC rem. (%)	Sturm test		OECD Screen. test DOC (%)	Closed bottle test BODT$_{30}$ (%)
			DOC rem. (%)	After days	DOC rem. (%)	BODT$_{14}$ (%)		CO$_2$ evol. (%)	DOC rem. (%)		
	FULL AGREEMENT IN ALL TESTS: RESULTS POSITIVE										
8	Adipic acid	99 ± 4	100	4	96	92	--	91	100	96	83
9	Dehydracetic acid	99 ± 9	96	6	91	77	--	63	100	98	81
10	Citric acid	93 ± 5^a	85	1	--	--	100	97	100	100	90
11	Alkylsulfonate	93 ± 5	--	--	--	--	90	97	91	98	73
12	n-Dodecylsulfate	107 ± 6^a	97	14	93	70	100	82	69	107	85
13	2-Aminobenzoic acid	103 ± 2	97	1	86	70	93	71	100	100	90
14	4-Aminobenzoic acid	98 ± 2	96	3	82	74	100	104	100	100	79
15	Aniline	98 ± 3	100	3	96	99	--	92	98	93	90
16	1,2-Dihydroxy-benzene	98 ± 4	98	4	88	80	--	62	96	91	89
17	4-Hydroxybenzoic acid	99 ± 5	100	1	88	98	--	61	91	95	93
18	3-Hydroxybenzoic acid	99 ± 2	94	2	92	93	--	93	99	40	90
19	4-Nitrobenzoic acid	91 ± 5	96	5	77	56	--	109	95	100	48

Table III-18. Biodegradability in Various Tests (Gerike and Fischer, 1979)

Compound No.	Compound	Coupled units test DOC rem. ± $Tol_{95\%}$ ($)	Zahn-Wellens test DOC rem. ($)	Zahn-Wellens test After days	MITI test DOC rem. ($)	MITI test $BODT_{14}$ ($)	AFNOR test DOC rem. ($)	Sturm test CO_2 evol. ($)	Sturm test DOC rem. ($)	OECD Screen. test DOC ($)	Closed bottle test $BODT_{30}$ ($)
	LOW RESULTS ONLY IN THE CLOSED BOTTLE AND/OR MITI TEST										
20	1-Methyl-pyrrolidone	99 ± 8	98	4	95	92	--	91	97	99	0
21	Benzylamine	101 ± 3	96	4	95	85	--	90	98	96	11
22	3-Nitrophenol	83 ± 11	84	13	93	56	98	60	92	95	2
23	Diethylene glycol	53 ± 13	95	7	24	20	59	31	33	42	80
24	Terephthalic acid	93 ± 3	93	4	0	0	--	72	91	82	112
25	2-Nitrobenzoic acid	98 ± 6	99	6	2	4	--	75	99	100	68
26	Diethanolamine	94 ± 3	94	3	0	3	--	88	97	100	10
27	4-Nitrophenol	100 ± 4	92	10	1	3	97	90	98	100	0
28	Triethanolamine	91 ± 3	82	8	0	2	97	91	100	96	0

Table III-18. Biodegradability in Various Tests (Gerike and Fischer, 1979)

Compound No.	Compound	Coupled units test DOC rem. ± Tol$_{95\%}$ (%)	Zahn-Wellens test DOC rem. (%)	Zahn-Wellens test After days	MITI test DOC rem. (%)	MITI test BODT$_{14}$ (%)	AFNOR test DOC rem. (%)	Sturm test CO_2 evol. (%)	Sturm test DOC rem. (%)	OECD Screen. test DOC (%)	Closed bottle test BODT$_{30}$ (%)
	DISCREPANCIES AMONG OTHER TEST RESULTS										
29	N-Methylaniline	102 ± 4	92	8	26	3	--			17	0
30	Pyridine	99 ± 2	97	6	15	11	--	46	92	0	0
31	2-Chlorobenzoic acid	93 ± 8	94	1	1	8	0	58 102	97 98	17	0
32	3-Aminophenol	94 ± 4	93	10	4	0	--	10 0	31 25	7	0
33	Pentaerythritol	96 ± 1	97	12	0	4	--	70	100	0	0
34	Tertiary butanol	33 ± 4	96	6	0	7	--	9	43	29	0
35	3-Chlorobenzoic acid	30 ± 8	85	7	0	8	18	0 93	32 92	4	0
36	2-Chloroaniline	5 ± 5	94	10	21	0	--	5	25	-7	0
37	Sulfanilic acid	7 ± 8	-2	14	0	30	--	0 66	9 97	16	0
38	3-Aminobenzoic acid	5 ± 6	108	6	90	60	92	31 97	57 99	25	11

Table III-18. Biodegradability in Various Tests (Gerike and Fischer, 1979)

		Coupled units test	Zahn-Wellens test		MITI test		AFNOR test	Sturm test		OECD Screen. test	Closed bottle test
Compound No.	Compound	DOC rem. ± Tol$_{95\%}$ (%)	DOC rem. (%)	After days	DOC rem. (%)	BODT$_{14}$ (%)	DOC rem. (%)	CO_2 evol. (%)	DOC rem. (%)	DOC (%)	BODT$_{30}$ (%)
					INHIBITORY MATERIALS						
39	2-Nitrophenol	61 ± 23	80	14	7	0	16	32	43	100	0
40	Hydroquinone	89 ± 5	66	14	86	70	29	82	97	59	81
41	TBS	41 ± 9[a]	27	14	0	0	15	12	32	13	10
42	LAS	73 ± 6	95	14	0	0	18	53	64	75	65
43	BDMDAC	83 ± 7	78	14	0	0	0	50	95	8	0
44	CTAB	109 ± 19 104 ± 6[b]	53	14	0	0	0	0	0	7	0

[a] COD removal

[b] At 6 hr mean retention time

compounds include chemicals whose biodegradation is probably strongly concentration dependent. The results of the MITI and AFNOR tests appear to be very strongly affected by these types of chemicals, which is not too surprising since they employ the highest test concentrations. The AFNOR test does have an inhibition test (glucose metabolism with or without the test chemical) which does not appear to function properly. Both the MITI and AFNOR tests do not distinguish between LAS and TBS (TBS is not an inhibitory compound--it is only included in the category because of structural similarity), the behavior of both of which is well understood in sewage treatment plants and the aqueous environment.

Because of the high concentrations used, these two tests do not ditinguish between a readily biodegradable compound and a persistent compound. Thus, the utility of the results from the tests is reduced, at least for the alkylbenzenesulfonate chemical ions.

From their study of the seven biodegradation test methods, Gerike and Fischer (1979) reached several conclusions about the individual test methods. These are summarized in Table III-19. These conclusions were based upon the assumption that the results from the OECD coupled units tests were "correct."

In a follow up study, Gerike and Fischer (1981) (1) tested 12 more compounds in the AFNOR test, (2) tested 15 compounds that had previously given variable results in an SDA semi-continuous activated sludge (SCAS) test that had been modified for measuring DOC removal, and (3) tested compounds that previously degraded in one test but not the other by using the first as a preacclimation step for the second. This study suggested no major changes from the evaluations reached in Table III-9. However, they did conclude that the SDA-SCAS test "exaggerated strongly with respect to the predictive value of biodegradability test results for the general environment." Even compounds that did

Table III-19. Evaluation of the Biodegradation Test Methods
(Gerike and Fischer, 1979)

Closed Bottle Test - economical, high capacity, safe evaluation in positive sense (positive probably right), high evaluations for strong concentration dependent chemicals, too stringent - should have trace elements, vitamins and preacclimation step added

Modified OECD Screening Test - yields environmentally relevant positives without being overly restrictive - test chemical concentration too high, needs preacclimation step

Sturm - surprisingly vigorous (may be too vigorous), reproducibility not always good

AFNOR - positive probably safe in an environmental sense, sensitive to chemicals that are strongly concentration dependent, inhibition test does not seem to work

MITI - positive safe in environmental sense, underrates surprisingly often, involved inoculum step does not fulfill purpose

Zahn-Wellens - vigorous, models industrial sewage treatment plant

OECD Coupled Units Test - good for evaluating biodegradability of chemicals that have toxicity problems or require special conditions; good model of an activated sludge treatment plant

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not degrade well in the OECD Coupled Units Test were degraded in the SDA-SCAS procedure.

Fischer and Gerike (1975) have also compared the biodegradation of a number of surfactants and detergent builders in the OECD Screening Test, the Closed Bottle Test, the OECD Confirmatory Test, and the OECD Coupled Unit Test. These results are summarized in Table III-20 and show general agreement between the four methods with the exception of NP9 and NP10 where the screening tests show little degradation but the continuous tests indicate appreciable breakdown.

The OECD (1980) has conducted an extensive ring test of the following six biodegradation methods with five test chemicals:

	Methods		Test Materials
1.	Modified OECD Screening (39)*	1.	4-Nitrophenol (4-NP)
2.	MITI Test (11)*	2.	Diethylene glycol (DEG)
3.	Closed Bottle Test (16)*	3.	Sodium tetrapropylenebenzenesulfonate (TPBS)
4.	Sturm CO_2 Test (11)*	4.	Aniline (A)
5.	Zahn-Wellens Test (24)*	5.	Sodium 4-acetylaminobenzenesulfonate (SAS)
6.	French AFNOR Test (7)*		

* Number of laboratories conducting the test

The aim of the program was to study the performance of the selected test methods and to find suitable biodegradability standard materials. The results obtained provide considerable insight into the intra- and interlaboratory reproducibility of the tests.

Figure III-25 examines the frequency of ranking of the individual chemicals from the results obtained by the different laboratories (5 is the "softest" or most biodegradable) (see key in upper left hand corner of Figure III-25). The OECD Modified Screening Test provides the following biodegradability ranking SAS/TPBS/4-NP/DEG/A (hard to soft), while in the other tests, TPBS is more frequently ranked "harder" than SAS. Results for 4-nitrophenol (4-NP) are scattered for all the tests and particularly for the Closed

Table III-20. Comparison of Biodegradation of Surfactants and Builders in Four Biodegradability Tests (Fischer and Gerike, 1975)

Sample	OECD Screening Test Anal. Loss %	Closed Bottle Test BOD$_{30}$ %	Closed Bottle Test Anal. Loss %	OECD Confirmatory Test Anal. Loss %	OECD Coupled Units Test COD or DOC Loss %[a]
TPS	< 35	0	~10	36	41.2 ± 9.4
LAS	~95	60-65	90-95	90-95	94.1 ± 4.6 69.6 ± 8.3 66.4 ± 5.1
Na-*n*-dodecylsulfate	99	~85	99	99	106.5 ± 5.7 96.1 ± 1.5
NP10	4[b] 87[c]	2	5	>90	68.5 ± 6.6 78.4 ± 4.1 69.4 ± 3.1 69.1 ± 6.4
NP9		1	10	97.4	49.4 ± 0.8 48.0 ± 5.9
n-o-NP-9	80	29	84	90.0	61.1 ± 6.9
n-C_{16}-fat alcohol +10 EO	99	71	100	98.2	53.6 ± 6.5 67.2 ± 6.1 56.4 ± 12.0
n-C_{16}-fat alcohol +30.7 EO	84	59	99	98.1	32.5 ± 6.2 33.1 ± 4.5
Polyglycol, MW600		70			69.1 ± 9.3 -5.9 ± 2.2 65.0 ± 10.0
Na_3 citrate		90			93.2 ± 4.6
Na_4-cyclopentane-tetracarboxylate		0			3.6 ± 5.3 -2.1 ± 13.2
Maleic acid-propylene cooligomer	0			32.4 ± 3.5	33.3 ± 7.4
Na poly(α-hydroxy-acrylate)		0-16			105.8 ± 8.9 94.8 ± 6.8 99.7 ± 4.5

[a] Various retention times, inoculum, and analytical methods

[b] 19 days soil inoculum

[c] 32 days sewage inoculum

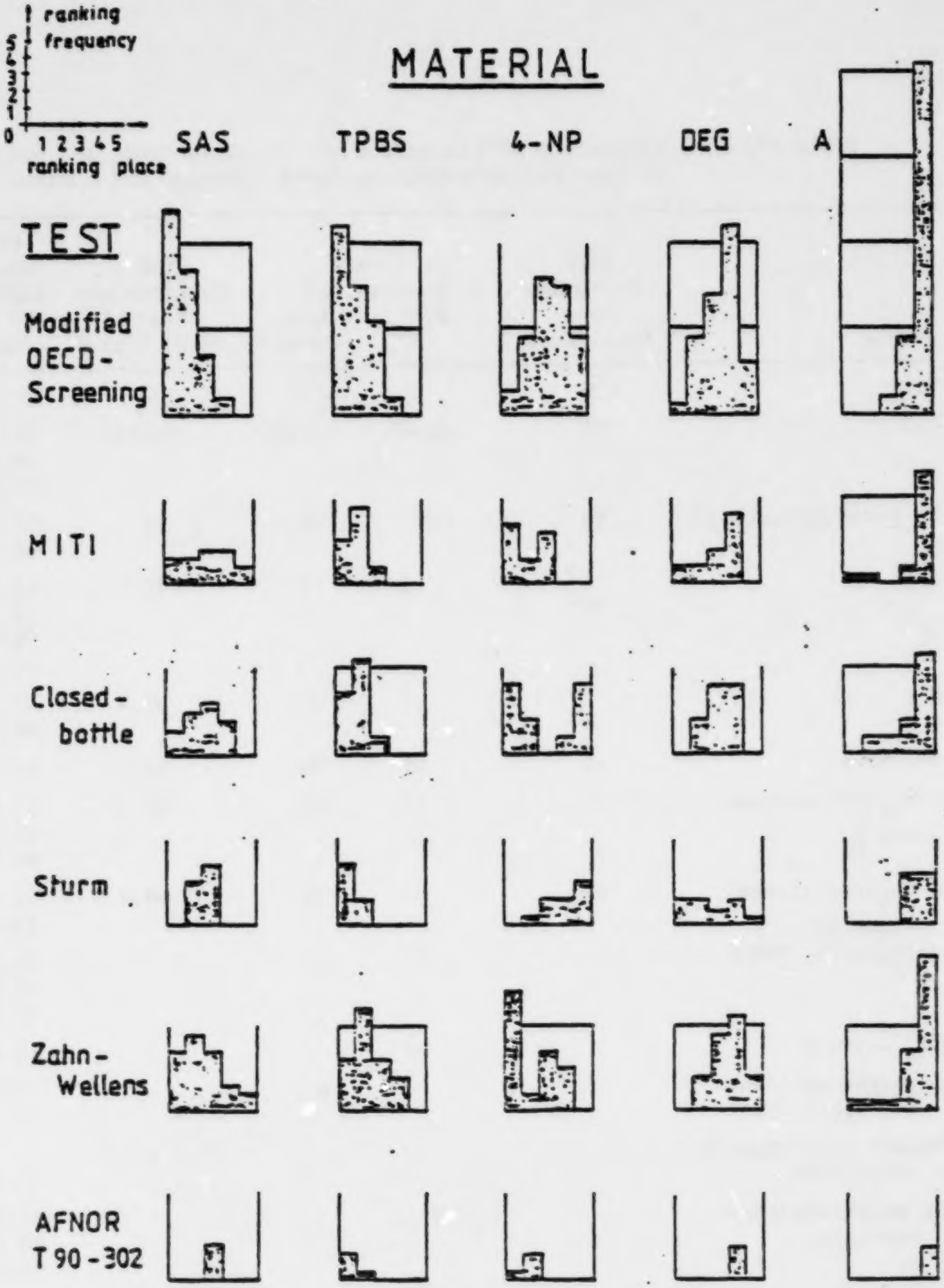

Figure III-25. Frequency of ranking on places from "hard" to "soft".

Bottle Test where some laboratories report it to be relatively biodegradable while others indicate it is more non-biodegradable.

Table III-21 presents the individual biodegradation percentages at arbitrary points chosen from the biodegradation curves. These results illustrate the considerable variation reported by different laboratories for the same test and same chemical. In a few instances where results were repeated by the same laboratory, the reproducibility of the method can be assessed. For example, using the OECD Modified Screening Test, one laboratory reported 96 percent and 15 percent degradation for 4-nitrophenol. In their final report, the OECD (1980) concluded that "at the actual state-of-the-art, no test method is sure for giving the same ranking of test materials in different laboratories." However, the results do suggest that using a variety of test methods will allow chemicals to be placed in approximate biodegradation potential categories.

Saeger and coworkers at Monsanto have used a variety of test procedures to examine the biodegradation of several chemical groups including adipic acid esters (Saeger et al., 1976), phthalic acid esters (Saeger and Tucker, 1976), and phosphate esters (Saeger et al., 1979). These results are summarized in Table III-22. In general, the Sturm results correspond in relative order of biodegradation to the SDA-SCAS test. For example, trixylenyl phosphate is one of the slowest degrading compounds in both tests. Also interesting to note is the poor reproducibility for the Sturm test with tributyl phosphate. This lack of reproducibility was also noted by Gerike and Fischer (1979). Comparison of the river die-away results to the other procedures is also interesting. The three slowest degrading phthalate esters in the river die-away test were also the slowest in the SDA-SCAS procedure. This is also generally true for

Table III-21. Biodegradation Results

Modified OECD-Screening Test (Percent biodegradation at 19th day as indicated by DOC reduction)

Laboratory Code	4-NP	DEG	TPBS	A	SAS
656	0	0	0	87	0
112	100	100	21	100	-
316	16;2	30;15	27;71;6	-;100	10;0
076	81	40	2	85	5
614	95	0	0	93	10
165	96;100	44;79	22;27;57	103	89
518	86	5	10	77	38
245	0;0;0	7;0	0;0	100;100	-
930	67	22	11;5	83	3
083	9	0	0	93	19
478	-	-	-	-	-
394	65	85	1	100	22
134	-	-	-	-	-
(405)	(-950);(-400)[a]	(-400)[a];-	(-437)[a];(700)	(?);(189)	(-67)[a];(50)
984	83	75	0	94	0
522	66	89	39	99	46
286	53	37	0	94	50
086	100	63	39	98	11
969	73	87	15	43	39
125	95	42	37	99	95
205	96;15	21	18	93	94;103
643	3	33	6	100	0
805	3	42	5	84	0
963	2	15	5	96	1
323	1	58	11	95	13
(222)	100	67	0;0	100	0
249	4	86	4;0	100	0
912	100	82	34	100	19
380	87	92	66	88	< 1
860	7	66	18	97	8
824	41	72	17	87	35
488	2;94	44	7	81	0
128	100	88	-	100	9
896	0	46	0	92	0
430				96;100	0;0
442	0	-	40	99	-

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[a] Negative values are comparisons to controls where less DOC was removed with the test compound than with the controls

Table III-21. Biodegradation Results (Cont'd)

AFNOR Test (Total percent biodegradation during 42 day test period as determined by DOC reduction)

Laboratory Code	4-NP	DEG	TPBS	A	SAS
165	90;94;16	90;97;94	27;(140?);-7	96;103;96	90;44;54
205	107	102	22	102	33
323	<0	79	0	94	28

MITI-Test (Percent biodegradation at 14th day from BOD measurement)

112	-	-	-	70	-
165	4;0;-2;-3;34;41;-3;-1;1';-1;-1;2;35;44;-2;-2;-2;1;-4;-1	0;0;5;4;79;8;3;5;0;2;3;17;8;7;-22;10;0;2;0	-1;0;-1;0;0;-1;-1;0;0;0;0;0;0;-6;-7;-1;-1;0;1;3;0;0;0;-3;-3;0;0;-1;3	38;38;46;44;42;40;36;45;45;45;43;43;47;46;39;41;42;41;38;41	4;1;3;-1;0;-1;-1;3;1;0;-1;1;1;1;1;-1;0;2;0;1
518	-3	42	-5	127	19
125	82	3	11	88	87
205	0	8	2	84	0
963	67	0	0	70	82
(222)	0	2	0	75	0
860	-2	45	-4	92	-7
824	-11	45	2	93	14
488	0	6	0	94	5
128	0	9	0	82	11
896	0	7	0	84	8

MITI-Test (Percent biodegradation at 14th day from DOC reduction)

112	-	-	-	94	-
165	;93;95;0;4;0;6;-2;-2;43;43;4;-9;-6;-6	20;82;-27;-50;3;0;0;3;13;11;7;54;0;0;6	3;-1233;-1200;3;3;5;7;7;0;-5;8;-18;30;0;7;10;14;0;32;-7;0;0;6	-40;-60;94;94;93;89;90;90;92;57;0;93;96	0;2;5;3;3;2;-2;0;0;-8;-8;21;0
518	-2	15	19	93	15
125	100	3	14	-	95
205	2	12	1	84	0
(222)	14	0	-	90	5
860	2	83	10	97	5
824	-5	50	8	99	-2
488	2	6	9	93	1
128	1	15	5	99	19
896	0	1	0	98	1

Table III-21. Biodegradation Results (Cont'd)

Closed-Bottle-Test (Percent biodegradation at 30th day from BOD measurement)

Laboratory Code	4-NP	DEG	TPBS	A	SAS
316	0	31	24	116	(98?)
614	0	9	4	85	4
165	109	45	19	68	0
930	0	54	0	75	0
478	0	78	34	48	52
394	65	43	6	47	20
134	56	81	18	87	99
984	91	30	< 2	96	< 2
969	79	102	30	43	76
205	95;(98)	33;19	1;1	82;(70)	44;26
643	8	6	1	7	7
(222)	77	83	22	113	37
380	19	83	70	87	72
896	0	8	0	115	62
430	84	37	0;11	92	41

Sturm-Test (Percent biodegradation at 26th day from CO_2 evolution measurement)

086	36;34	17;19	9;10	86;92	30;29
205	86^{4};85^{3}	35;55	5;26	112^{5};192	90^{12};130^{16}
643	88;89	11;17	19;19	78;83	75;25
963	79;50	5;24	4;18	82;50	58;30
323	75;6	52;86	3;33	83;63	59;42
249	101;161	56;103	20;46	98;103	39;90
896	69;68	73;54	11;7	89;71	42;27
430	78;61	56;75	<0;<0	85;89	85;9

Zahn-Wellens-Test (Percent biodegradation at 19th day from DOC reduction)

656	40	0	64	99	0
076	-6	100	57	98	29
614	0	99	79	96	0
165	-2;97;-4	91;100	(14?);21	105	106
518	25;13;23;16	98;99;100;100	64;78;28;34	99;100;-	41;29;100;99
245	67;92	98;100	0;0	100	46
522	94	99	65	100	45
286	94	100	0	100	47
125	12;95	96;95	52;56	102;102	103;99
205	100;100	98;100	26;(16);28	99;98	91;98
643	98	98	66;64	99	26;22

Table III-21. Biodegradation Results (Cont'd)

Zahn-Wellens-Test (Percent biodegradation at 19th day from DOC reduction) (Cont'd)

Laboratory Code	4-NP	DEG	TPBS	A	SAS
963	95	100	27	99	22
323	96	99	<0	93	93
912	7	100	82	100	43
380	-	-	-	-	-
860	90	100	46	103	99
824	-69	100	23	99	95
488	0	81;92	3	100	82
430	-	-	-	100	83
442	93	-	2	100	-

Zahn-Wellens-Test (Percent biodegradation at 19th day from COD reduction)

Laboratory Code	4-NP	DEG	TPBS	A	SAS
165	1;112;1	95;101	36;34	106	107
522	94	99	61	100	31
205	88;94	99;99	36;(30);36	95;96	92;97
963	89	99	14	99	7
380	13;7	80;79	85;89	99	20;20
430	13;7	80;79	-	95;100	70;100

Table III-22. Biodegradation Test Results for Adipic Acid Esters, Phthalic Acid Esters, and Phosphate Esters (Saeger *et al.*, 1976, 1979; Saeger and Tucker, 1976)

	River Die-Away Test (% Remaining) days			Sturm CO$_2$% Theoretical	Gledhill CO$_2$% Theoretical	SDA-SCAS % Biodegrad.
	1	7	21			
Triphenyl phosphate	60-100	-[a]	NM[b]	81.8[c]		93-96
Tricresyl	95	-	NM	82.1[c]		97-99
Trixylenyl				43.8[c]		13-65
Cresyl Diphenyl	90	-	NM	84.5[c]		82
Isopropylphenyl diphenyl	90	40	15	48.8[c]		35-49
t-Butylphenyl diphenyl	90	20	NM	89.8[c]		84-93
Isodecyl diphenyl	95	65	0	63.3[c]		20-50
2-Ethylhexyl diphenyl	80	5	0	82.3[c]		74
Dibutyl phenyl	98	0	-	75-85[c]		52-95
Tributyl	100	0	-	3.3-91[c]		56-96
LAS	95	25	-			99+
Phthalic acid[d]	98-100	90-100	15-50			99+
Monobutyl phthalate						99+
Butylglycolyl butyl	70	0	-			99+
Butylbenzyl	70	2	-	95		93-99
Di-ethylhexyl	100	95	70	86		70-78
Di(heptyl, nonyl, undecyl)	100	100	75	86		48-54
Diundecyl	100	95	55			29-45
p,p'-DDT						-7
Dextrose				96.4	93.8	
Di(heptyl, nonyl)adipate				90.2	78-82	67-88
Di(2-ethylhexyl)				93.8	81.6	73-92
Di-*n*-hexyl				90.2	81.2	95-99
1,3-Butyleneglycol adipic acid polyester				88.1	78.1	

[a] Not detectable

[b] Not measured

[c] 28 days

[d] Measured by UV

the slowly degrading diphenyl aryl phosphates (isophenyl, _t_-butyl, and isodecyl).

Some other investigators have provided data that can be used for comparisons of different methods. For example, Yordy and Alexander (1980) studied the breakdown of N-nitrosodiethanolamine (NDEIA) in sewage and in lake waters using radiolabeled material. They found that NDEIA was not mineralized in the two lake waters examined but was mineralized in sewage (Figure III-26) (used radiolabeled NDEIA). Although NDEIA was not mineralized in lake water, it was metabolized as indicated by analytical procedures that measured intermediate metabolites. This result suggests that the microorganisms necessary for mineralization are absent from lake waters but present in sewage. Larson (1979) also compared the results from his CO_2 evolution test with removal by the SDA-SCAS Test for a number of surfactants. The only discrepancy was with a surfactant that appeared to absorb to the sludge in the SDA-SCAS test, resulting in physical removal.

3. Biodegradation Kinetics

Development of microbial kinetics for environmental biodegradation has only recently been attempted, mostly by investigators at the EPA Athens Laboratory (Lewis _et al_., 1975; Paris _et al_., 1975; Wolfe _et al_., 1978; Baughman _et al_., 1980) and SRI International (Smith _et al_., 1978). Theoretical bases for support of microbial kinetics have been drawn from enzyme kinetics (Michaelis-Menten equation - White _et al_., 1964; Wolfe _et al_., 1978) or from microbial growth kinetics (Monod equation - Baughman _et al_., 1980). In either case, the equation derived is the same although the assumptions that have to be made are different.

The Monod kinetic equation (Monod, 1949) can be used to describe the relationship between substrate concentration and rate of cell growth.

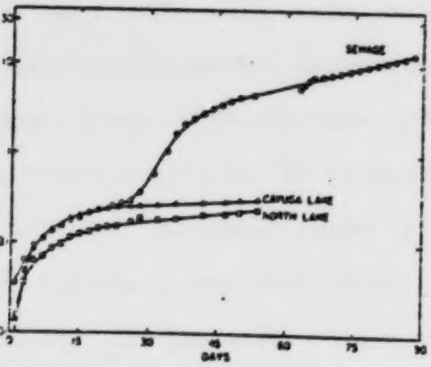

Figure III-26. N-Nitrosodiethanolamine mineralization in samples of lake water (Reprinted from Yordy and Alexander, 1980. Microbial metabolism of N-nitrosodiethanolamine in lake water and sewage. Appl. Environ. Microbiol., 39(3):559-565, with permission from the American Society for Microbiology.)

$$\frac{d[B]}{dt}\,\frac{1}{B} = \mu = \frac{\mu_m S}{K_S+S}$$

[B] = microbial population or activity per unit volume

μ = specific growth rate

μ_m = maximum specific growth rate

S = concentration of growth limiting substrate

K_S = concentration of substrate supporting half maximum growth rate (0.5 μ_m)

By adding a yield coefficient ($Y = -d[B]/d[S]$) which describes the efficiency of substrate conversion to biomass, it is possible to describe kinetics based upon substrate transformation, but requires the assumption that the growth yield from a substrate is constant. If one is concerned about low level input of the substrate into the environment, the concentration of the substrate can be assumed to be much less than K_S (K_S values typically range from 0.1 to 10 mg/ℓ - Baughman et al., 1980) and the equation can be reduced to the familiar form of second-order kinetics (This may not be a safe assumption for many chemicals since environmental concentrations of a few to 100 ppb are not uncommon.):

$$-\frac{d[B]}{dt}\,\frac{d[S]}{d[B]} = -\frac{d[S]}{dt} = \frac{\mu_B[B][S]}{Y(K_S+[S])}$$

$$-\frac{d[S]}{dt} = \frac{\mu_m}{YK_S}[B][S] = K[B][S]$$

"The constants μ_m, K_S, and Y are dependent on the characteristics of the microbes, pH, temperature, other nutrients, and some other environmental conditions, and are based on the rate of utilization of a growth rate-limiting single substrate by pure microbial cultures" (Chou, 1980). Thus, the rationale for using a second-order rate expression for mixed cultures, and in situations where the substrate is not the only carbon source, is tentative (Baughman et al., 1980) and raises many questions. The possibility of a threshold at low concentrations has been suggested (Boethling and Alexander, 1979a). The process of acclimation has been ignored in these rate expressions and is assumed to already occur before the rates are measured. For a system receiving substrate input over a long period of time, this may or may not be reasonable, depending upon whether the acclimation process for the substrate is reversed under different environmental conditions (temperature, nutrients, etc.). For example, if acclimation to a substrate never occurs at a low temperature (there are many examples of this; see Howard _et_ _al_., 1975) then the rate of degradation measured in the laboratory at room temperature will never be realized. Also, the relationship of the "lag" time to the overall period of degradation in which one is interested is important. For example, a lag period of 2 days can be important if the model covers only 4 days, but probably is not so important if the model covers longer periods of time.

Another question that arises is how to measure the biomass of the active microbe(s). In pure cultures this is relatively simple but pure cultures are poor simulators of nature (see Section III.A.5). With mixed cultures, plate counts have been used in river die-away systems and adenosine triphosphate (ATP) measurements have been used in aerobic sediment systems (Baughman _et_ _al_., 1980). Any method will be acceptable as long as it is adequately reproducible and comparable from one site to another but it should be specific for the population of microorganisms that are active on the chemical or reproducibly correlate to their population. Use of the Monod equation also assumes that the growth yield is related in a constant manner to degradation (Larson, 1980). Larson (1980) cites an example of Ohio River water where 90 percent of an alkyl ethoxylate is converted to CO_2 and only 10% of the radiolabel was incorporated into the cells, suggesting that high growth rates are not a requirement of degradation.

The Michaelis-Menten equation for enzyme reactions also has been used for theoretical development of biodegradation kinetics. This approach requires slightly different assumptions.

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

V_{max} = maximum velocity of substrate uptake

$[S]$ = substrate concentration

K_m = Michaelis-Menten constant = substrate concentration at which $V = 1/2\ V_{max}$

Assuming a constant ratio of enzyme activity to microbial biomass, the substrate removal can be expressed as:

$$-\frac{d[S]}{dt} = \frac{V_{max}[S]}{K_m + [S]}[F]$$

$[F]$ = mg (dry weight) of microbe per liter

At substrate concentrates much lower than K_m the equation reduces to a second order equation (Wolfe _et_ _al_., 1978).

$$-\frac{d[S]}{dt} = k[S][F]$$

Again, this equation only applies to pure culture systems and requires the assumption that there is a constant ratio between enzyme activity and biomass. Application of this equation to environmental biodegradation kinetics with mixed cultures requires similar considerations to those discussed above. In addition, it requires the assumption that passage of the substrate through the microbial cell membrane is not rate determining.

Only a limited number of studies on a limited number of compounds have undertaken the development of microbial kinetics. The early studies were conducted mostly with pure cultures. For example, Lewis et al. (1975) studied the kinetics of biodegradation of malathion with a fungus isolated from a pond. By rearranging the Michaelis-Menten equation to the following equation (Lineweaver-Burk equation)

$$\frac{[S]}{V} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}}$$

and plotting experimental values of [S]/V (mg malathion removed per hour per mg fungus) and [S] (mg malathion per liter solution), values were determined for V_{max} of 7.8 $\pm$ 2.6 x 10^{-3} mg malathion per hour per mg fungus and K_m of 16.3 $\pm$ 6.7 mg/ℓ malathion. Biomass of fungus was determined by dry weight. Comparison to a bacterial strain indicated that the transformation velocity for the bacteria was 5000 times greater than for the fungus. Using similar procedures, Wolfe et al. (1978) determined the fungal kinetics of Propham and Chloropropham. Bacterial kinetic rate constants on these pesticides taken from published values were two orders of magnitude higher than fungal rates.

Smith et al. (1977) used four procedures to develop biodegradation rates with mixed cultures as well as cultures isolated by enrichment culture systems:

- Batch fermentations with low-level inocula of washed biodegrading cells [shake flasks - enrichment (mixed) cultures washed in phosphate buffer first]
- Continuous chemostat fermentations (exponential phase enrichment culture added to sterile chemostat)
- Cascade batch fermentation (start with enrichment cultures followed by daily sequential transfers to new flasks containing fresh water samples, salts, and substrate)
- Batch fermentations with large microbial populations and low substrate levels (enrichment culture seed)

> "If an enrichment culture that could degrade the substrate as a sole carbon source was not developed within six weeks, no metabolite or kinetic studies were carried out" (Smith et al., 1977).

For the first three procedures, V_{max} and K_m were determined and then the rate constants were calculated. With batch fermentations with large microbial populations and low substrate, the second order rate constant becomes pseudo-first-order.

$$\frac{d[S]}{dt} = \frac{k'[S]}{b}$$

In this case, the second order rate constant can be calculated by dividing the pseudo-first-order rate constant by the biomass.

$$k = \frac{\frac{k'}{b}}{[B]}$$

These procedures have been used with eleven chemicals to develop kinetic data (Smith et al., 1978).

Each of the procedures has advantages and shortcomings. The cascade batch fermentation is most similar to nature (natural microbial cultures are added daily) but requires daily water sampling and if realistic low levels of substrate were used, it would be impossible to determine the biomass attributed to the metabolism of the compound (Smith et al., 1977). The batch fermentations with low microbial populations and continuous culture methods are labor intensive (Chou, 1980) and could suffer from changes in relative microbial populations in the culture mixture (Smith et al., 1977). The latter is probably realistic to expect in nature although it does not provide for good kinetics. Also, in these two procedures, if realistically low substrate concentrations are used, biomass determinations would be subject to considerable error (Smith et al., 1977). Chou (1980) has recommended the use of batch fermentations with large microbial populations to develop biodegradation kinetics because it (1) requires less time, (2) needs no significant changes in total number or relative components of organisms, and (3) is mathematically simple to calculate. Of the eleven chemicals studied by Smith et al. (1978), only one (methyl parathion) was studied with all four techniques. The second-order rate constants from the various procedures are listed in Table III-23. Considering the diversity of techniques, the agreement is fairly good suggesting that batch fermentations with high microbial populations and low substrate levels might be an easy way to provide second-order rate constants that normally would require considerable labor. However, this correlation has only been shown for this one compound.

Larson (1980) has suggested that microbial growth kinetics (Monod equation) cannot be used to predict biodegradation kinetics because of a lack of a constant growth yield and because measuring growth in environmental systems is difficult, especially when concentrations of chemicals are low. He has suggested measuring the rate of decrease of the chemical as a function of substrate concentration in situations of high biomass and low substrate levels where pseudo-first-order kinetics prevail. In addition, he has recommended the use of CO_2 evolution rather than substrate disappearance because of its simplicity, even though it may give rate constants that are a little lower than the true value. This approach is basically similar to that recommended by Chou (1980).

From the above review, it can be seen that there are several ways to develop biodegradation kinetics in the laboratory, but whether the values obtained have any relevance to biodegradation kinetics in nature is questionable. Most of the studies avoid the question of acclimation by using pure cultures or enriched cultures that have already been acclimated to the test substrate in developing the kinetic values, although Larson (1980) has added a lag period factor to the equation he suggests for developing pseudo-first-order

Table III-23. Second-Order Rate Constants for Methyl Parathion Determined by a Variety of Procedures

	ml cell^{-1} hr^{-1}
Batch Fermentation with Low-Level Inocula of Washed Biodegrading Cells	1.3×10^{-7}
Continuous Chemostat Fermentation	7.7×10^{-7}
Cascade Batch Fermentation	0.46×10^{-7}
Batch Fermentation with Large Microbial Population and Low Substrate Level	0.2×10^{-7}

kinetics. The Larson (1980) equation simply subtracts the lag period that is noted in the laboratory experiment, which may or may not be a good simulation of what occurs in nature. Both of the theoretical approaches to microbial kinetics require assumptions that are tenuous when it comes to applying them to natural systems. They both really only apply to pure culture systems where the substrate is the only carbon source. The enzyme approach requires the assumptions that the transport of the substrate through the cell membrane is not rate determining and that the enzyme to biomass ratio is constant. With the microbial growth kinetics approach, one has to assume a constant growth yield. The use of high microbial populations with low substrate levels to develop pseudo-first-order kinetics has considerable appeal due to the ease with which it can be conducted experimentally. However, if enrichment culture or other manipulations are used to develop the seed for the kinetics, the microbial population, which probably will not change during the procedure, will be very artificial and not very representative of a natural population.

At the EPA Athens Laboratory, several microbial transformation kinetic studies have been conducted with mixed populations (Paris et al., 1979; Paris et al., 1980; Wolfe et al., 1980) found in natural water samples without any acclimation. During these studies, natural waters from various sites were selected without prior consideration of water quality. The temperatures of the water samples at the time of collection ranged from 1½ to 27½C and upon receipt in the laboratory water quality parameters (pH, TOC, N, P, hardness, bacterial concentration) were measured and major genera of bacteria determined (Paris et al., 1979). The bacterial concentration was monitored by plate counts and the pollutant concentration by gas chromatography; the studies were conducted at the temperature of water when sampled. Malathion, butoxylethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), and chlorpropham (CIPC) were studied at concentrations of less than one milligram per liter and in most cases, the bacterial population did not increase during the test period (pseudo-first-order with respect to pollutant). The mean values of the second order rate constants were $(5.2 \pm 3.9) \times 10^{-10}$, $(4.5 \pm 2.8) \times 10^{-11}$, and $(2.8 \pm 1.2) \times 10^{-14}$ ℓ organ^{-1} hr^{-1} for 2,4-DBE, malathion, and CIPC, respectively (Paris et al., 1979). No significant (95% confidence level) temperature effect on the rate constants was observed in the range 1° to 27°C (which seems unusual but was not addressed by the authors). Using similar experimental procedures, Paris et al. (1980) determined mean values of oxidative second order rate constants of $(1.6 \pm 1.2) \times 10^{-11}$ and $(2.7 \pm 0.96) \times 10^{-11}$ ℓ organ^{-1} hr^{-1} for phenanthrene and o-chlorotoluene, respectively. Second-order biolysis rate constants for a series of phthalate esters has also been determined (Wolfe et al., 1980) with these experimental procedures.

The driving force for developing microbial kinetic data has been its use in environmental fate modeling efforts. It still remains to be seen whether the kinetics developed in any of the ways used by SRI provides any better data than just placing the chemical in natural water or water-sediment system and examining the rate of disappearance of the chemical. Considerably more work needs to be conducted here. The number of chemicals that have been studied is very limited and needs to be increased, especially chemicals that are not likely to undergo enzymatic hydrolysis (a considerable number of the compounds studied so far and the correlations developed - Wolfe et al., 1980 - have been for chemicals that can hydrolyze). Also, the effects of low substrate concentration (Boethling and Alexander, 1979a) and external carbon sources need to be addressed, as well as the development of a perspective on how acclimation processes should be handled.

4. General Conclusions on Aqueous Biodegradation Test Procedures

Considerable research and data have been generated since our last report in 1975. Large numbers of chemicals have been tested in a variety of test procedures and considerable insight into the accuracy and reproducibility of the results has been gained. However, these results, especially the work of Gerike and Fischer (1979) and the interlaboratory comparison testing of the OECD (1980), do not give one a great deal of confidence that a result obtained in one test will be similar in another or that the result with a test in one laboratory will be similar to the result with the same test in another laboratory. In fact, the OECD (1980) indicated that the state-of-the-art is such that no test is sure of giving the same ordering of chemicals in different laboratories. With the 44 chemicals examined by Gerike and Fischer (1979) only 19 were in full agreement in all the tests and 10 chemicals had discrepancies in all the tests, and all these tests were conducted in the same laboratory.

It appears that biodegradation tests do fairly well for chemicals that are on either end of the persistence spectrum (either very non-biodegradable or very biodegradable). However, for chemicals that are at neither extreme or are otherwise unique (e.g., inhibitory chemicals) the results are scattered.

For a new chemical that needs to be tested, there does not appear to be any one test that can be used that will provide considerable confidence that the material is biodegradable or non-biodegradable. Probably the best approach would be to test it with several procedures. Each of the test procedures that has been examined in some detail has some advantages and some disadvantages. The use in screening tests of analytical procedures that are nonspecific to the test chemical certainly is advantageous to screening large numbers of chemicals, since a new analytical procedure does not have to be developed for each chemical. However, as indicated in the more detailed discussion on analytical methods, these indirect analytical methods have some disadvantages also. For example, BOD and CO_2 evolution can be used with most any type of chemical (volatile, water insoluble), but only give an indication of the energy of metabolism which may or may not correlate well with a chemical's degradation. COD does not have the latter problem but has nitrite interference problems and poorly determines some chemicals (e.g., pyridine does not oxidize under COD conditions). DOC can only be used with water soluble, non-volatile chemicals, may require high levels of test compound, and gives an all or nothing result (mineralized or not).

Defined mineral salts media aqueous biodegradation tests have been used for basically two reasons:

1. assumption that the more one defines the media and the microbial seed, the more reproducible will be the result (assume more reproducible than, for example, river water)
2. allow for the use of indirect analytical procedures

From the results of Gerike and Fischer (1979) and the OECD (1980), the first of these is in considerable doubt. However, whether the use of natural water or water-sediment samples would be any more reproducible is questionable, but needs to be studied.

Some progress has been made on further understanding the factors that affect biodegradation results and extrapolation to nature (e.g., test chemical concentration -- Boethling and Alexander, 1979a,b), but considerably more research is needed here. For example, it is assumed by many investigators that if the microbes can acclimate to a chemical under one set of conditions in the laboratory, that this will occur in the environment. This reasoning is used by Baughmann et al. (1980) to suggest that chemicals that are continuously released to the environment will have acclimated microbial cultures in nature that will be able to degrade the chemical at a rate similar to that determined in the laboratory. This is not necessarily so since it has been shown that temperature affects acclimation processes and may never allow acclimation to occur in nature. Other effects that need to be examined are pH, particulate matter (for insoluble compounds), organic content, etc.

The possibility of biodegradation kinetics being developed for modelling purposes has only been considered in the last several years and was not covered in our previous report. As covered in detail in Section III.D.3, there are several theoretical and practical problems to be overcome. Either theoretical approach (enzyme or growth kinetics) requires assumptions that apply to pure culture systems containing a single carbon substrate and may not be appropriate for environmental systems which contain mixed cultures and multiple carbon sources. On the practical side, there is no really good way to measure the biomass of the population of microorganisms that are active on a test substance.

There are many questions that still need to be resolved and it is possible that these answers will only be forthcoming after a larger data base (more chemical groups tested in a variety of natural and defined test systems) has been generated from which to evaluate various kinetic expressions, whether or not they have any direct theoretical support. Also, the effects of acclimation, inoculum, temperature, pH, substrate concentration, external carbon, etc. on the kinetic rates need to be determined.

E. Cost Analysis

In the following, the cost analysis that was presented in the 1975 report has been repeated along with accompanying text with the exception that the costs have been inflated from 50 to 300% to represent the increases in material and labor that have occurred since 1975. In addition, there have been some cost analyses that have been published since 1975 that are presented for comparison purposes. For example, some of the screening tests that have been reviewed by

OECD have had cost estimates in 1978 Swiss Francs. These costs have been converted to U.S. dollars at the exchange rate prevailing in January 1981. Also, the Pesticide Office of EPA has published estimates of the costs likely to be incurred for the environmental chemistry tests that are necessary for pesticide registration.

For cost purposes, the techniques used to study the environmental persistence and degradation of chemicals in water systems have been divided into four sections. The first section (1a) consists of preliminary or screening tests which provide information on the relative degradability of materials. The second section (1b) provides costs for intensive studies of metabolic pathways and breakdown product identification. The third section (2) discusses the dynamic techniques used for fairly detailed studies of biodegradation under sewage treatment conditions. The fourth section (4) reviews the recent cost estimates for a variety of tests that have been made by OECD or the EPA Office of Pesticides Programs (OPP).

A major cost factor in biodegradation studies is that of the analytical equipment. In prediction of the cost, it has been assumed that an average life of small equipment (e.g., shaker, spectrophotometer, oxygen meter, etc.) is approximately five years (220 working days/year), whereas that of other capital equipment (e.g., scintillation counter, gas chromatograph, etc.) is ten years.

The equipment costs for a test have been estimated on the basis of the number of days required to perform the test, assuming that the instrument will be occupied all that time. The cost for each test is calculated for one compound and for twenty compounds. The labor cost for the test is calculated at the rate of $100/day (MS level worker, including benefits). The overhead is assumed to be 125% of the professional services.

1. Techniques for Studying Biodegradation of Chemicals in Water

a. Preliminary Test to Determine Biodegradability

The cost estimates given in Table III-24 are more appropriate for a new laboratory, which is interested in undertaking biodegradation studies. These estimates do not represent the price of the test quoted by a commercial laboratory which is engaged in testing biodegradability on a routine basis. For example, the 5 day BOD costs are 5 to 10 times what a commercial laboratory would charge.

The results of the preliminary screening test will be helpful in deciding the necessity and approach to be used in more intensive studies. For example, if the preliminary test indicates low biodegradability, the intensive study might use an enrichment culture technique to determine if any breakdown can be detected. If moderate degradation is noted in the preliminary test, the intensive study may concentrate on metabolite identification.

b. Biodegradation Study to Identify Metabolites and Elucidate Pathway of Degradation

Once the biodegradability of a compound has been established, a detailed study can be undertaken to elucidate biochemical pathways and characterize the nature of intermediate metabolites formed. For this purpose, pure cultures of microorganisms have generally been considered more suitable. Some researchers have utilized cell-free preparations in these studies; however, the results thus obtained are of very little ecological or environmental relevance. This phase of biodegradation testing is time consuming and expensive and, therefore, should be undertaken only with those compounds for which preliminary evidence for accumulation of an intermediate metabolite has been obtained. For example, the CO_2 evolution or BOD is substantially lower than theoretically possible. In the intensive study, radiolabelled material is generally used, which may sometimes have to be custom synthesized. Organic synthesis of a number

Table III-24. Cost Analysis for Preliminary Biodegradability Test

Biodegradation Test Method	Analytical Method	Analytical equipment needed and their cost	Equipment cost for the test	Chemicals glassware and misc. supplies	Labor cost $100/day	Overhead 125% of professional services	Total cost	Cost/ compound	Remarks
5 or 10 day BOD (1)	Chemical analysis	--	--	85	100	125	310	310	
5 or 10 day BOD (20)	Chemical analysis	--	--	525	500	625	1650	85	
5 or 10 day BOD (1)	Oxygen meter	Oxygen meter $800	Negligible	75	75	95	240	240	
5 or 10 day BOD (20)	Oxygen meter	Oxygen meter $800	Negligible	420	200	250	870	45	
Two bottle-single dilution-reaeration method (1)	Chemical analysis	--	--	500 (includes 4 biodegradation units)	200 ($150 if oxygen meter is used)	250	950	950	
Two bottle-single dilution-reaeration method (20)	Chemical analysis	--	--	3500 (includes 40 biodegradation units)	1200 ($600 if oxygen meter is used)	1500	6200	310	
Two bottle-single dilution reaeration method (1) of Buzzell et al. (1968)	Carbon analysis Chemical analysis	Nitrite determination apparatus (colorimeter) $675; High speed centrifuge $6500; Carbon analyzer $5000	90	275	1000	1250	2615	2615	Following parameters are studied in this test: Biochemical oxygen demand, Total carbon, Bacterial population Nitrification
Two bottle-single dilution reaeration Method of Buzzell et al. (1968) (20)	Chemical analysis Carbon analysis	Same as above	600	3500	7000	8750	19,850	990	

Table III-24. Cost Analysis for Preliminary Biodegradability Test (Continued)

Biodegradation Test Method	Analytical Method	Analytical equipment needed and their cost	Equipment cost for the test	Chemicals glassware and misc. supplies	Labor cost $100/day	Overhead 125% of professional services	Total cost	Cost/ com- pound	Remarks
Warburg respirometry (1)	Warburg manometer	Warburg respirometer $3400; Centrifuge $6500	10	85	200	250	545	545	
Warburg respirometry (20)	Warburg manometer	Same as above	40	875	1000	1250	3165	160	
Oxygen electrode respirometer (1)	Polarography	Oxygen polarography $1500; Recorder $2000; Temperature control unit $650; Centrifuge $6000	15	85	100	125	325	325	
Oxygen electrode respirometry (20)	Polarography	Same as above	70	575	600	750	1995	100	
River Die-Away Test (1)	Chemical analysis	Colorimeter $675	Negligible	85	150	185	420	420	Labor cost could vary from compound depending on the complexity of the chemical method of analysis
River Die-Away Test (20)	Chemical analysis	Colorimeter $675	Negligible	850	1000	1250	3100	155	
River Die-Away Test (1)	Gas chromatography	Gas chromatograph $12,000	15	85	200	250	450	450	
River Die-Away Test (20)	Gas chromatography	Gas chromatograph $12,000	90	1225	1200	1500	4015	200	
River Die-Away Test (1)	U.V. Absorption	Spectrophotometer $2000; Centrifuge $6500	10	75	100	125	310	310	
River Die-Away Test (20)	U.V. Absorption	Same as above	60	525	500	625	1710	85	

Table III-24. Cost Analysis for Preliminary Biodegradability Test (Continued)

Biodegradation Test Method	Analytical Method	Analytical equipment needed and their cost	Equipment cost for the test	Chemicals glassware and misc. supplies	Labor cost $100/day	Overhead 125% of professional services	Total cost	Cost/ com- pound	Remarks
Shake culture test (1)	Colorimetry	Shaker $1200; Colorimeter $675	Negligible	85	150	45	420	420	
Shake culture test (20)	Colorimetry	Shaker $2000; Colorimeter $675	40	1225	1000	1250	3515	175	
Shake culture test (1)	Gas chroma- tography	Shaker $1200; Gas chromatograph $12,000	20	85	200	250	555	555	
Shake culture test (20)	Gas chroma- tography	Shaker $2000; Gas chromatograph $12,000	120	1225	1200	1500	4045	200	
Shake culture test (1)	Radioactive material $^{14}CO_2$ measured or parent compound	Scintillation counter $18,000	20	1400 (includes labelled material)	200	250	1870	1870	
Shake culture test (20)	Same as above	Same as above	230	21,000 (includes labelled material)	2500	3125	26,855	1345	
Shake culture test (1)	CO_2 evolution	--	--	130	100	125	355	355	
Shake culture test (20)	Same as above	--	--	1225	800	1000	3025	150	
Shake culture test (1)	Growth measure- ment	Spectrophotometer $2000	Negligible	85	100	125	310	310	
Shake culture test (20)	Same as above	Same as above	Negligible	785	700	875	2360	115	

Table III-24. Cost Analysis for Preliminary Biodegradability Test (Continued)

Biodegradation Test Method	Analytical Method	Analytical equipment needed and their cost	Equipment cost for the test	Chemicals glassware and misc. supplies	Labor cost $100/day	Overhead 125% of professional services	Total cost	Cost/ compound	Remarks
Shake culture test combined with oxygen utilization and succinic dehydrogenase activity assay (1) (Buzzell et al., 1969)	Carbon analysis manometry; succinic dehydrogenase by colorimetric method	Freeze drying apparatus $5000; Warburg respirometer $3400; Centrifuge $6000; Carbon analyzer $5000; Colorimeter $675	70	175	500	625	1300	1300	Following parameters are studied in this test: carbon removal oxygen utilization, succinic dehydrogenase activity
Same as above for 20 compounds	Same as above	Same as above	850	1225	6000	7500	15,575	780	
Model ecosystem (1)	Radioactive material	Scintillation counter $18,000	75	1225 (includes radioactive material needed for thin layer chromatography, auto radiography)	800	1000	3100	3100	
Model ecosystem (20)	Same as above	Same as above	750	8750	8200	10,250	27,950	1400	

(The number in parenthesis indicates the number of compounds tested.)

of suspected metabolites may be needed in order to identify the unknown metabolites of the test chemical. The cost estimate for intensive study of biodegradation is as follows:

One Compound

Labor - Ph.D. level (20k) Six months	$10,000
Overhead	12,250
Equipment - GC-Mass Spec. (have analysis performed by a commercial lab) Scintillation counter (cost for six months = $750)	4,000
Chemicals, glassware Miscellaneous supplies - material for thin-layer and paper chromatography, autoradiography, etc.	1,100
Total Cost	$27,600

Twenty Compounds

Labor - Ph.D. level (20k) five years*	$100,000
Overhead	125,000
Equipment - GC-Mass Spec. (cost $150,000, depreciate over 10 years; cost for 5 years, $75,000) Scintillation counter, cost for 5 years $4,500.	78,500
Chemicals, glassware and misc. supplies	10,000
Total Cost	$314,500
Cost/Compound	$15,725

* The labor cost assumes a detailed study of four compounds per year.

2. Techniques Which Simulate Sewage Treatment Plant Conditions

The costs for the major continuous and semi-continuous techniques are detailed in Table III-25. The costs of the screening tests have already been reviewed in Table III-24. The cost of the combined shake culture-Warburg

Table III-25. Cost Estimates for Techniques Used to Simulate Sewage Treatment Plant Conditions

Biodegradation Test Method*	Analytical Method	Analytical Equipment Needed and Cost	Test Apparatus Costs	Chemicals, Glassware, and Misc. Supplies	Labor Cost $100/day	Overhead 125% of Profess. Services	Total Cost	Cost/ Compound
Official German Activated Sludge Test (1)	MBAS	Colorimeter $675	750	750	(7 weeks) 3500	4375	10,050	10,050
Official German Activated Sludge Test (20)	"	Colorimeter $675	3750	7500	14,000	17,500	43,425	2171
Miniaturized continuous activated sludge								
Sweeney and Foote, 1964 (1)	MBAS	Colorimeter	750	150	(9 days) 900	1125	3600	3600
(20)	"	"	3000	3000	6300	7875	20,850	1042
Swisher, 1967a,b (1)	"	"	300	375	(30 days) 3000	3750	8100	8100
(20)	"	"	1200	3000	12,000	15,000	31,875	1594
Trickling filter (1)	MBAS	Colorimeter	450	750	(9 weeks) 4500	5625	12,000	12,000
(20)	"	"	2700	7500	18,000	22,500	51,375	2569
SDA, 1965 Semi-continuous activated sludge (1)	MBAS	Colorimeter	150	150	(20 days) 2000	2500	5475	5475
(20)	"	"	1500	1500	8000	10,000	21,675	1084

* The number in parenthesis indicates the number of compounds tested.

respirometry screening technique used by Buzzell et al. (1969) to study shock loading should be somewhat less than the sum of the individual techniques.

The cost estimates in Table III-25 used the same analytical technique so that the difference in the total cost per compound is only a reflection of the procedure. The analytical procedure can drastically affect the total cost, the magnitude of which is indicated previously in Table III-24. The costs also demonstrate that continuous techniques are, for the most part, more time consuming and, therefore, expensive. The Official German Test referred to in Table III-25 is the OECD Confirmatory Test.

3. New Cost Estimates by EPA and OECD

In Table III-26 are summarized the cost estimates provided for the protocols that have been evaluated by the OECD. The MITI estimate is extremely high due to the labor estimate which is based upon 1 person x 18 days x 8 hours/day = 144. Obviously, many chemicals can be tested in that time frame so the labor probably should be considerably reduced.

The OECD (1980) also conducted rings tests in a number of laboratories using six methods and five chemicals. From these tests, estimates of labor and cost for each of the tests were provided by the participating laboratories (Table III-27).

The cost estimates of the EPA Pesticide Office for conducting environmental chemistry tests according to the protocols required for registration are tabulated in Table III-28. Most of these tests require the use of radiolabelled chemicals. These cost estimates compare fairly well with the "intensive biodegradation study" estimates. Upon reflection, the updated 1975 report costs for chemicals seem unrealistic, since radiolabelled chemicals usually cost between $5 to $10,000. The EPA cost estimate for the SDA-SCAS test

Table III-26. Cost Estimates of Test Protocols Evaluated by the OECD

Test	Equipment and Material (1978 Swiss Francs)	Labor (person-hours)	1978 Swiss Francs	1978 U.S. Dollars	Reference
Closed Bottle Test	12,700	7	650	416	OECD (1979a)
Sturm CO_2 Evolution	40	18 (minimum of 3 test chemicals)	2000 (1979) (assumes 1 man-hr with overhead = 100)	1200 (1979)	OECD (1980)
Modified OECD Screening Test	55-69,000	10	750-1000	480-640	OECD (1979c)
French AFNOR Test	25,000	12	700	448	OECD (1979d)
Zahn-Wellens Test	46,000	12	900	576	OECD (1980)
Japanese MITI Test	33,000	144	8000	5120	MITI (1979)
SDA-SCAS	28,000 (1979)	8	28,700 (1979)	17,220 (1979)	OECD (1980)
OECD Confirmatory Coupled Units Test	46,500	150	12,000	7680	OECD (1980)

Table III-27. Labor and Costs for a Variety of Aqueous Biodegradation Tests (OECD, 1980)

		Mod. OECD Screening	MITI Test	Closed Bottle Test	Sturm CO_2	Zahn-Wellens	AFNOR
Labor (per test chemical)							
< 10 person-hours	no. of	5	2	6		2	
10-19 person-hours	labs	9	5	5	4	5	3
> 19 person-hours	reporting	12	1		2	8	
Cost/dollar/test material centered around		500	600	200	400	700	?

Table III-28. Cost Estimates for Environmental Chemistry Tests Requirement by EPA for Pesticide Registration (U.S. EPA, 1978b)

	Cost per Test
Aerobic Aquatic	$ 25,000
Anaerobic Aquatic	$ 25,000
Effects of Microbes on Pesticides	$ 28,000
Activated Sludge (SDA-SCAS)	$ 15,000

is considerably higher than the updated 1975 report value, partially due to the use of radiolabelled chemicals.

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IV. BIODEGRADATION OF CHEMICALS IN THE SOIL ENVIRONMENT

The chemicals found in soil result either from direct application or indirect application. For the most part, pesticides provide the largest source of direct application. Indirect application results from spray fall-out, from rainfall and dust, from industrial discharge into air and subsequent settling down to soil, from translocation by animals and plants, and from flooding of polluted water on soil. Indirect application can be a major factor in contributing to widespread soil contamination by synthetic chemicals. Only a portion of the chemicals found in soil results from direct application. For example, many soils that have never been treated with DDT contain small amounts of DDT; presumably the chemical comes from spray drift or atmospheric fall-out.

Soil can be viewed as a complex system that consists of chemical reactants and chemical and biochemical catalysts. The biochemical catalysts are composed of free-state and absorbed enzymes as well as living cells. Microorganisms seem to be the major contributor to the degradation of chemicals in soil. Chemical reactions and photoreactions (only to a very shallow depth) may also contribute to degradation. This section will evaluate the existing methods used since 1975 in determining the biodegradation of synthetic chemicals in soil. In many instances, methods have not significantly changed; however, they are included to illustrate their application to a wide variety of chemicals in the soil environment. Thus, the organization of this section will not be significantly different from that of the 1975 report (Howard et al., 1975). In addition, this report does not represent an exhaustive search of the literature, but rather a sampling of the state-of-the-art.

Since the 1975 report, a number of soil metabolism tests have been proposed and required by the United States Environmental Protection Agency. Many of these protocols have been or are in the process of being evaluated and validated. Some soil degradation methods are also being considered by the Organization for Economic Cooperation and Development (OECD) and their recommendations will be discussed in Section C.2.

A. Techniques Used for Determining Biodegradation

The existing methods for determining biodegradation in soil can be generally classified into three categories. They include laboratory tests, greenhouse studies, and field studies. Field studies reflect the fate of the chemicals under actual environmental conditions. Laboratory tests are carried out under a controlled environment and they are often time saving, convenient and low cost; whereas greenhouse studies are between laboratory tests and field studies in terms of environmental simulation and complexity. The laboratory tests cover natural communities from soils, pure culture studies, cell-free extract studies, and miscellaneous methods.

Quite often researchers have used sterile soil control along with non-sterile soil tests to distinguish between chemical and biological degradation. Controls are also used to distinguish physical, photochemical, or chemical removal and degradation of the chemical from biological degradation. The commonly used methods of soil sterilization for this purpose are autoclaving (normally for 60 to 120 minutes at 121°C and 15 psi for 3 to 6 successive days, Fleeker _et_ _al_., 1974; Gowda and Sethunathan, 1976; Schooley _et_ _al_., 1975; Oliver _et_ _al_., 1979), ethylene oxide sterilization in a vacuum desiccator for 2 to 3 days (Oliver _et_ _al_., 1979), sodium azide treatment (100-500 ppm [parts per million], Pramer and Bartha, 1972; Wahid _et_ _al_., 1980), or gamma irradiation

(^{60}Co exposure for 1 hour at 2-6 Mrad, Helweg, 1979; Rosenberg and Alexander, 1980a,b; Wahid et al., 1980).

The form in which the chemical is added can influence its degradation (Ou et al., 1978; Ahmad et al., 1979a,b). In most instances the chemical is added as the technical grade (>95 percent purity); however, the test chemical if it is a pesticide can also be added to soil as a formulation which may consist of the salt of the test chemical and inert ingredients.

1. Laboratory Tests

a. Natural Communities from Soil

The techniques discussed in the following section rely on the use of microorganisms occurring in natural soil. Soils collected from the field are treated with test chemicals in the laboratory. The soils are normally stored in polypropylene bags at room temperature to retard moisture loss at 0°C, or at -20°C (Ahmad et al., 1979a). The treated soils may be incubated under (a) aerobic conditions (open air or forced aeration) or (b) anaerobic conditions (flushing with nitrogen or other inert gas and/or flooding the soil). To achieve further anaerobiosis, a readily degradable substrate may be added. The treated soils may be mixed with water or aqueous solutions containing mineral salts and/or organic substrates and incubated under stationary or perfused conditions, or if an aqueous suspension is used the suspension may be shaken.

i) Soils Incubated with Test Chemicals

This testing method has been widely used. Soil samples are well mixed with the test chemicals and incubated under stationary conditions with or without flushing with air, O_2 (for aerobic conditions); or flooding, N_2 or $N_2 + CO_2$ (for anaerobic conditions). In this regard, this method is more closely related to the natural systems than the other laboratory testing methods.

Soils collected from the field are generally dried in air and then passed through a sieve. These soils, in many studies, are subjected to a physicochemical analysis including pH, particle size distribution, percentage of organic matter, ion exchange capacity, total nitrogen content, total carbon content, water-holding capacity, etc.

(a) Aerobic Studies

The majority of the degradation studies are carried out under aerobic conditions. Although the methods for maintaining aerobic conditions (soil samples left open to the air, soil samples covered but left with air space, and soils flushed with air) were reviewed in the 1975 report, certain modifications of these methods warrant mentioning. Soil samples placed in Erlenmeyer flasks and sealed with rubber stoppers were opened to the air every day for 15 minutes (Ahmad et al., 1979b). The rubber stopper can be split and resealed around a stem (Hsu and Bartha, 1979). A syringe needle was used to flush air through traps to maintain aerobic conditions. The top of the container can also be sealed with a ground-glass jointed reflux column to trap the volatile test chemical and carbon dioxide (see Section B.1.d.iii) with aeration by diffusion (Anderson and Domsch, 1980).

(b) Flooded Conditions

For simulating flooded conditions (such as in rice fields), water is generally added to air-dried soil. Complete anaerobiosis can be achieved by the addition of a biodegradable substrate as well. No new pertinent data were found.

(c) Anaerobic Conditions

Strictly anaerobic conditions can be achieved by flushing the soil systems (flooded or unflooded) with inert gases such as argon and nitrogen or with hydrogen (Marinucci and Bartha, 1979b) or a mixture of hydrogen and carbon dioxide. Martens (1978) studied degradation of ^{14}C-diclofop-methyl under a N_2 atmosphere in flooded soils. Hackett et al. (1977) studied $^{14}CO_2$ and $^{14}CH_4$ production from ^{14}C-lignin compounds in vials sealed with serum stoppers and purged with N_2. Smith et al. (1979) demonstrated the degradation of pendimethalin and its derivatives in flooded soils (under a N_2 atmosphere). A manifold assembly system was constructed to study degradation of ^{14}C-labeled pentachloronitrobenzene in anaerobic soils (Murthy and Kaufman, 1978). The nitrogen was scrubbed of CO_2 and saturated with water before passing over the soil. A polyurethane filter trapped volatile ^{14}C-products (see Section B.1.d.iii.a and Figure IV-3).

ii) Soils Suspended in Aqueous Solution

No new pertinent data were found.

iii) Soil Perfusion Technique

Discussion on and diagrams of the soil perfusion apparatus are covered in the 1975 report.

Soils used for perfusion experiments are dried in air, screened through a sieve, and may be mixed additionally with other components such as sea-sand (Kurane et al., 1978) to provide further support in the soil column. Soil samples may be treated with unlabeled compound (Fung and Uren, 1977) or labeled compound (Fournier, 1974) either before starting perfusion or they may be perfused with aqueous solutions containing the test chemical. The amount of soil employed in a perfusion experiment may vary from 10 to 50 g (air-dried basis), and 200 to 250 ml of distilled water or dilute solution containing a test chemical is often used for perfusion. In most cases, the concentration of test chemical under study is in the range of 5 to 100 ppm. Samples of perfusate can be taken from the reservoir at assigned intervals of time, or daily, for analysis of the chemical residue and degradation products. The rate of perfusion varies according to the investigator. The method requires either that the parent compound or a product of interest appears in the perfusate.

b. Pure and Mixed Culture Studies

i) Pure Cultures Isolated from Soil Enrichments

In order to show soil microorganisms are involved in the degradation of natural and synthetic chemicals in soils, and to establish routes of breakdown it is helpful to isolate microorganisms in pure culture from soils. This demonstrates that a particular microorganism or some combination of microorganisms may be degrading a particular chemical or structurally-related chemicals. The most effective and the most commonly employed method for isolating the effective microorganisms is by means of soil enrichment culture techniques. The basic principle behind the techniques consists of increasing the population of the effective microorganisms in the soils or in soil suspensions by treatment with the test chemical.

(a) Pure Cultures Obtained by Treatment of Soil with Test Chemical

Although no new data on this subject were found since the 1975 report, it is worth repeating the technique involved in obtaining pure cultures from soil enrichments. Soil suspensions of the treated soil are incubated under stationary conditions or on a rotary shaker. After incubation for a period of time, aliquots of the mixtures are transferred for incubation into fresh liquid medium containing the test compound. The incubation temperature is generally controlled at room temperature or at the temperature from which the soil sample was taken.

The growth of microorganisms can be judged from the increase in turbidity of the enrichment culture medium, or by microscopic observations. That microorganisms are active in the enrichment cultures can be shown by measuring the decrease in the concentration of the test chemical. For example, this can be easily determined by release of chlorine ion from chlorinated hydrocarbons (Rosenberg and Alexander, 1980b), appearance of degradative products, or by a decrease in UV absorption of aromatic compounds. For the isolation of an effective microorganism(s) from the enrichment cultures, agar plates of the mineral salt medium containing the same test chemical are streaked with the enrichment culture fluids normally diluted with distilled water or buffer (Raymond et al., 1976). The isolates are purified by repeated streaking on the same medium or on a rich medium such as nutrient agar. Each pure culture is then tested for its ability to degrade the appropriate chemical in an appropriate medium (Cook et al., 1978; Clark et al., 1979). The active microorganism is generally maintained on agar containing the appropriate chemical or occasionally in rich medium such as nutrient agar. However, maintenance on nutrient agar can cause loss of the degradative ability of the culture. A pure culture may also be obtained by diluting untreated soil and plating aliquots of the dilution on agar plates containing various concentrations of the test chemical. Each pure culture is then tested for its degradative ability as mentioned above.

(b) Naturally Enriched Cultures

This soil enrichment technique involves using natural soils which have been treated with the test chemical to obtain effective microorganisms without further subcultures. No new pertinent data were found in the literature.

(c) Soil Perfusion Cultures

Another soil enrichment technique uses soil perfusion to enrich the effective microorganisms. The technique of soil perfusion has been mentioned previously (A.1.a.iii). Isolation of pure cultures of effective microorganisms from the perfused soils is accomplished via a soil dilution plate method. The soil perfusion technique has also been used to isolate bacteria that degraded di-2-ethylhexyl phthalate (DEHP) (Kurane et al., 1978).

ii) Mixed Cultures Isolated from Soil Enrichment

Studies concerning the degradation of synthetic chemicals can be carried out using mixed cultures of microorganisms from soils. From the point of view of obtaining information relating to the biodegradation of chemicals in the soils, it is desirable to use mixed populations of soil microorganisms since such a system more closely approximates the natural soil ecosystem. In a mixed population, different microbial species possessing diverse metabolic activities may act synergistically in degrading a chemical and are, therefore, more likely to metabolize a greater variety of compounds.

The most common technique for obtaining a mixed microbial population is to flood a soil sample in an Erlenmeyer flask. The liquid may be distilled water, an inorganic salts medium with or without the test chemical, or the medium supplemented with either accessory growth factors such as yeast extract or vitamin B complex, and/or a readily degradable carbon source. The soil slurry is then shaken for 60 to 120 minutes at the desirable temperature (normally 25-30°C). Shaking is normally done on a wrist shaker or reciprocal rotary shaker. After the allotted time, the slurry is allowed to settle and the liquid layer removed. The liquid layer is then treated with the test chemical, aerated, and incubated. At intervals, portions of the culture fluid are withdrawn for assay of chemical residue and/or metabolic products. The environmental parameters vary considerably depending upon the nature of the experiment. Kilpi (1980) studied the degradation of phenoxy acid herbicides by mixed cultures isolated from soil treated with 2-(2-methyl-4-chloro)phenoxypropionic acid. Rosenberg and Alexander (1980a) reported a mixed suspension of microorganisms obtained from 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)-treated Philippine soil degraded 2,4,5-T and 2,4,5-trichlorophenol (2,4,5-TCP) in a nutrient-supplemented medium. Miles and Moy (1979) reported the degradation of endosulfan by a mixed soil population. Clark et al. (1979) studied the degradation of polychlorinated biphenyls by a mixed microbial culture.

c. Cell-free Extract Studies

In order to provide further indication that the degradation of compounds is due to enzymatic reactions, cell-free extracts are used. For example, cell-free extracts can also provide information for establishing the pathway of intracellular degradation (Daughton et al., 1979a; Rast et al., 1979) and to determine if degradative enzymes are constitutive or induced (Cook et al., 1978; Rosenberg and Alexander, 1979). Degradative enzymes have also been isolated directly from soil (Getzin and Satyanarayana, 1979). Normally sterile and boiled controls are carried out to determine non-enzymatic formation of degradative products or disappearance of test chemical. The activity of the enzyme system is normally reported as substrate disappeared or product formed per minute per mg of protein (determined by a standard protein assay method). The preparation of cell-free extracts was reviewed in the 1975 report.

d. Terrestrial Model Ecosystems

A number of terrestrial model ecosystems similar to the aquatic and terrestrial-aquatic ecosystems (see Section III) have been developed to study the environmental fate of various chemicals. Cole et al. (1976) used a 19 liter wide-mouth glass jar fitted with an air inlet and outlet for air sampling and maintained in a plant growth chamber. In each ecosystem, 50 corn seeds were planted in vermiculite or loam soil and sprinkled with 2 ℓ of reference water containing trace minerals. The following invertebrates were added at various times after pesticide application: 10 earthworms, 10 garden slugs, 12 pillbugs, and 10 saltmarsh caterpillars. One prairie vole was also added to the system 15 days postplanting.

Gile and Gillet (1979) have also used a terrestrial labora tory ecosystem with which they have examined the environmental fate of several fungicides. The chamber consisted of a glass box (1 m x 0.75 m x 0.61 m) with a plexiglass lid that received a 16-hour daily photoperiod and was maintained at temperatures ranging from 30.25°C during the day to 18.25°C at night. The soil medium was 10% (by weight) Jiffy Mix Plus, 45% 20-grit sea sand, and 45% illite clay. The flora and fauna consisted of plants (alfalfa and perennial ryegrass), invertebrates (bacteriophagic nematodes, earthworms, pillbugs, mealworm larvae, gray crickets, and garden snails), and vertebrates (one gravid gray-tailed mole).

Nash and Beall (1980) and Nash _et_ _al_. (1977) have reported the use of microagroecosystem chambers for studying the fate of fungicides and insecticides (Figure IV-1). Their chambers were enclosed in glass (150 x 115 x 50 cm) and were equipped for air circulation for cooling and collection of volatilized pesticide. Inlet and outlet air were filtered through polyurethane foam. The soil was a sandy loam (pH, 6.7; organic matter content, 5.2%; 15.6%, soil moisture) that was fertilized. Five 19-day-old plants were transplanted into each chamber and sprayed with pesticide.

e. Miscellaneous Methods

The 1975 report reviewed the construction and use of a lysimeter. Studies of the degradation of oil sludge (Dibble and Bartha, 1979b) and dicamba (Glass and Edwards, 1979) in lysimeters have been reported. The degradation of chemicals under various environmental conditions with respect to pH, organic matter concentration, and temperature has also been studied in lysimeters (Dibble and Bartha, 1979a).

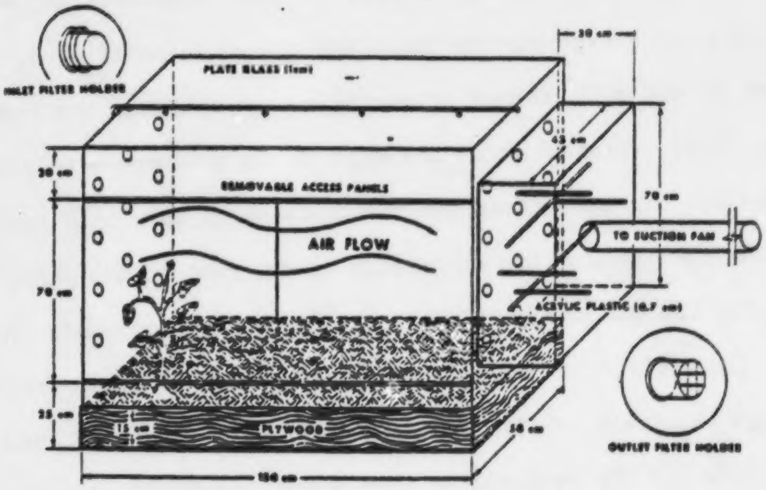
INLET FILTER HOLDER
PLATE GLASS (1cm)
30 cm
43 cm
30 cm
REMOVABLE ACCESS PANELS
70 cm
AIR FLOW
TO SUCTION FAN
70 cm
ACRYLIC PLASTIC (0.7 cm)
25 cm
15 cm
PLYWOOD
58 cm
OUTLET FILTER HOLDER
156 cm

Figure IV-1. Diagram of agroecosystem chamber (Nash et al., 1977).

2. Greenhouse Studies

A limited number of researchers have studied biodegradation in soil under greenhouse condition. In this test, soils obtained from the field are treated with various concentrations of test chemicals before being placed in containers, or conversely the soils are placed in containers, then test chemical applied to the soil either by mixing or by spraying the chemical on the soil (Beall, 1976). Treated soils are kept in the greenhouse in order to observe the degradation. In most cases the chemicals which have been tested are herbicides including phenoxyalkyl and carboxylic acids, benzoic and phenylacetic acids, oil sludge (Dibble and Bartha, 1979a), or ^{14}C-labeled compounds (Ebing and Schuphan, 1979). The toxicity of the chemical in the soils to plants can be assessed using seedlings (Kaufmann, 1977), roots (Otto _et al_., 1977), or whole plants (Nash and Beall, 1980). The response of the plant, seedling, or root to the chemical (i.e., bioassay) can be related to the herbicide residue in the soil. Soil treated with herbicide is watered to maintain adequate moisture. Ebing and Schuphan (1979) have developed a closed cultivating system for studying the behavior and degradation of chemicals in soil.

3. Field Studies

Most field studies have been focused on the degradation of organophosphate insecticides, chlorinated hydrocarbon insecticides, and chlorinated phenoxyalkyl carboxylic acid herbicides. Some of these compounds, such as DDT and aldrin, can persist in soil for greater than 10 years (Wilkinson _et al_., 1964).

In most studies the field is divided into plots. Each plot receives the test chemical with concentrations generally similar to actual field application. Two kinds of plots in terms of test surface area are employed; the so-called microplots (Walker and Smith, 1979) and regular plots (Banks _et al_., 1979; Beall, 1976). The plots may be block shaped (Banks _et_ _al_., 1979) or cylindrical rings (Berard and Rainey, 1979). Plots are separated individually by ridges of soil or by cultivated strips that are built up around each plot to prevent lateral surface movement of the test chemicals.

The treatment of test chemicals in soil plots is generally carried out by applying the test chemicals evenly over the soil. The chemicals may then be incorporated thoroughly into the soil to a desired depth. In the case of microplots, test chemicals are uniformly mixed by hand with a rake. In most cases, test chemicals are mixed with soil to a depth up to 15 cm (Banks _et_ _al_., 1979; McGrath, 1976) or more. The amount of test chemical applied to the soils is generally in the range of 2 to 10 pounds per acre, which is close to the actual dosage of many pesticides applied to crop fields. Higher concentrations are also used. In many cases, several different dosages may be applied to the soils to compare the persistence of the test chemical at various concentrations (McGrath, 1976). Such field experiments require adequate replication and untreated control plots. The chemicals can be applied to the soils only once or they may be applied annually for a few consecutive years. Soil samples are collected by means of a soil sampler, such as a Hoffer Soil Sampler (Berard and Rainey, 1979) or other device. If the vertical distribution of the chemical residues in the soil plots is to be studied, samples are collected from different depths (Banks _et_ _al_., 1979). Soil samples are then subjected to chemical analyses and/or biological assay.

B. Analytical Procedures

The precision and accuracy of the experimental results from soil degradation studies depend to a great extent upon the analytical methods employed. Good experimental setup without supporting suitable analytical proce dures can make the results meaningless. This is especially true in the case of degradation experiments of synthetic chemicals in the complex milieu of soil.

The analytical procedures used for determination of the chemical residues and their metabolic intermediates in soils can be generally divided into two categories: (1) chemical analyses and (2) bioassays. Chemical analyses cover chromatographic methods, spectrophotometric techniques, radioassays, CO_2 evolution, O_2 consumption, and GC-mass spectroscopic techniques. Bioassays include plant assays for herbicides and insect assays for insecticides.

1. Chemical Analyses

a. Extraction and Clean-up Procedures

Prior to analyses, chemical residues and their metabolites in soils have to be extracted from the soil samples and then concentrated. Soil contains a variety of inanimate materials which can react with both the parent and intermediary compound and thereby make their extraction extremely difficult. Devising a technique for extraction of an intermediate may be particularly difficult because the sorption and solubility characteristics of the intermediate may be unknown.

Most chemicals under study as well as most of their metabolites are organic in nature, and, therefore, organic solvents are commonly used for extraction. The choice of organic solvent depends on the chemical nature of the test compounds and their metabolites. Very often the solvent consists of a mixture of two or more solvents and the system chosen is the one that gives the highest recovery. For example, acetone and ethylacetate were used for removing phenoxyalkanoic acids and products (Rosenberg and Alexander, 1980a,b) and organophosphorous insecticides (Rosenberg and Alexander, 1979) from soil. Hexane, acetone, and methanol mixtures have been used to extract hexachlorobenzene from grass and soils (Beall, 1976). Phorate and terbufos were extracted from soils with chloroform and methanol (Ahmad et al., 1979a). Extraction can take place using hand shaking, mechanical shakers (Yoshida and Castro, 1975), Waring blenders (Berard and Rainer, 1979), sonication (Helling and Krivonak, 1978), or Soxhlet extractors (Beall, 1976; Raymond et al., 1976). The extract may be concentrated to an appropriate volume in a Kuderna-Danish evaporator-concentrator (Beall et al., 1976; Smith, 1979a,b) and then directly used for chromatographic analysis (Berard and Rainey, 1979), ion-exchange analysis (Nash and Bell, 1980), or it may be derivatized before analysis (Byast and Hance, 1975). In other cases, the extract needs further purification and/or clean-up in order to avoid unnecessary interference for chromatographic analyses. One method of clean-up requires that the concentrated extracts be purified by passing through a Florisil column (Beall, 1976), while another method is to pass the extract through a silica gel column (Raymond et al., 1976).

The problems involved in separation of the parent chemicals and their degradation products in the culture solutions of pure culture or mixed culture studies are generally less complicated and simpler. Unlike soil degradation studies, the chemical residues in the liquid medium of the pure culture studies are generally not associated with soil particles and are, therefore, easier to extract. By and large, the main techniques for separation of the chemical residues and their intermediates from the culture fluids are about the same as the separation procedures used with soil samples. In that case, the culture solution is extracted with solvent, and the extracts are dried with anhydrous sodium sulfate and then concentrated (Miles et al., 1979; Byast and Hance, 1975; Rosenberg and Alexander, 1979; 1980a,b). Clean-up may be required for some extracts. Furthermore, the culture solutions before being subjected to solvent extraction may have the hydrogen ion concentration adjusted to the appropriate pH by adding NaOH or HCl so that extraction efficiency is enhanced (Englehardt and Wallnofer, 1978; Hsu and Bartha, 1976; Nash and Beall, 1980).

In most cases, the degradation products in both the culture solutions or soil samples are by no means completely separated by solvent extractions. This is particularly true of many water soluble products.

b. Chromatographic Methods

Chromatographic methods have been widely used for separation and determination of parent chemicals and their degradation products in soil. Among them, gas-liquid chromatography, high-pressure liquid chromatography (HPLC), and thin-layer chromatography are most extensively used.

i) High-Pressure Liquid Chromatography

HPLC is a technique that is increasing in popularity. The technique is described in some detail since it was not covered in the 1975 report. Briefly, this technique employs a narrow column packed with a material that has a different affinity for each component of a sample dissolved in the liquid passing through the column. The separation of these components is correlated with the affinity of the column. The use of small packing particles (generally 5 to 10 µm in diameter) in small columns (10 to 25 cm long with inside diameter of 5 mm) coupled with the application of high-pressures (1000 to 6000 psi) and specially designed pumps to provide a constant flow of solution through the column (generally 1 to 3 ml/min) permits efficient separation of components. The solution is either one solvent (isocratic) or a mixture of solvents (gradient elution). Besides the column and pump, the other essential parts are the detector and recorder. Of the three types of detectors - refractive index, ultraviolet absorbency, and fluorescence - the UV detector offers a compromise between sensitivity and selectivity. The recorder receives impulses from the detector and plots these impulses as peaks of the various components of the sample injected. Advantages of HPLC are ease of sample preparation (generally no extraction necessary), size of sample (1 to 100 µℓ), speed, ability to collect intact components for further analyses, applicability to nonvolatile, thermally-unstable compounds, and size range of compounds (100-several million daltons). When combined with such accessories as a radioactive flow detector, HPLC provides both chromatographic and radiolabeled analyses. Selim and Cook (1978) used HPLC to identify residues of a dioxane herbicide in soil and soybeans, while Smith et al. (1979) identified degradation products of pendimethalin in anaerobic soil with HPLC.

ii) Gas-liquid Chromatography

When the chemical under study and some of its metabolic intermediates are volatile, thermally stable, and not very polar, gas-liquid chromatography (GLC) provides a good tool for quantitative determination. In most cases, a small volume (a few microliters) of the extract is injected into the instrument. Some of the chemicals or their metabolites that are relatively nonvolatile can be converted to volatile derivatives by simple procedures such as esterification. Since GLC is one of the most common analytical tools, there are numerous references to its use with many classes of chemicals. Table IV-1 provides a partial list of the chemicals, extracting solvents, types of GLC detector, and references associated with GLC analysis. Electron capture (ECD) and flame ionization detectors (FID) are the most commonly employed.

iii) Thin-layer Chromatography

No new pertinent data were found.

iv) Paper and Column Chromatography

No new pertinent data were found.

c. Spectrophotometric Methods

Spectrophotometric determination of synthetic chemical residues and their degradative products in soils usually does not have the sensitivity of gas-liquid chromatographic and thin-layer chromatographic analyses. It may not be able to distinguish between the parent chemical, metabolites, and degradation products. Nevertheless, it can be used for quick and easy determination of parent chemicals and their degradation products soils or it can be used as a confirmatory technique with GLC or TLC. There are three types of spectrophotometry often used in soil studies: UV (200 to 400 nm), visible (400 to 700 nm), and infrared (700 to 15,000 nm).

i) UV Absorption

No new pertinent data found.

ii) Visible Spectrophotometry

No new pertinent data found.

iii) Infrared Spectrophotometry

No new pertinent data found.

d. Radioassays

Radiotracer techniques have been a most useful tool for determining intermediary metabolism and detoxication mechanisms. The studies of the breakdown pathways of the chemicals (mainly pesticides) in soils are no exception. Radioassay usually can be achieved at nanogram levels of the labeled compound, and the specificity of analysis depends largely on the degree of separation and clean-up before scintillation counting or radioautography. Chemicals in soil studies are generally labeled with ^{14}C because of the required long test period, sensitivity of the method, and the detection of $^{14}CO_2$ evolution which is a good indication of mineralization. The selection of the site for labeling of a molecule is very important and depends upon what information one

Table IV-1. Gas Chromatography Assay of Chemicals

Chemical	Extracting Solvent	GC Detector	Reference
Fonofos, phorate, terbufos	Chloroform, benzene methanol	N-P-FID[a]	Ahmad et al., 1979a
Carbofuran	Acetone	N-P-FID	Ahmad et al., 1979b
Diallate	Benzene	^{63}Ni-ECD[b]	Anderson and Domsch, 1976
Fluoridone	Acetonitrile	^{63}Ni-ECD	Banks et al., 1979
Hexachlorobenzene	Hexane, acetone, methanol	^{63}Ni-ECD	Beall, 1976
Mirex	Acetone, hexane	^{3}H-ECD	Carlson et al., 1979
Polychlorinated biphenyls	Hexane	^{63}Ni-ECD	Clark et al., 1979
Parathion	Acetone, hexane	FID	Daughton and Hsieh, 1977
Alkylphosphonates, alkyl- and aryl-phosphates	Carbon disulfide, ethyl acetate, MEK	FID, P-FPD[c]	Daughton et al., 1979a,b
Kerosine, n-alkanes	Hexane	FID	Dibble and Bartha, 1979a,c
2,4-D, 2,4,5-T, 2,4,5-TP	Diethyl ether	FID	Dimock, 1975
Methomyl	Hexane, ether	FID	Fung and Uren, 1977
Dicamba	Diethyl ether	^{63}Ni-ECD	Glass and Edwards, 1979
Ametryn	Ethyl acetate, chloroform	Electrolytic conductivity	Goswami and Green, 1975
Dinitroaniline herbicides	Benzene, acetone	^{3}H-ECD, FID	Helling, 1976
Gossyplure	Heptane	FID	Henson, 1977
Guthion, parathion, pyrimiphos-methyl	Chloroform, methanol, hexane	FID	Heuer et al., 1976
Parathion	Acetone, ether, hexane	P-FPD	Katan et al., 1976
Lindane (hexachlorocyclohexane)	Benzene, methanol	^{63}Ni-ECD	Kohnen et al., 1975
Di-2-ethyl hexyl phthalate	Hexane	FID	Kurane et al., 1978

Table IV-1. Gas Chromatography Assay of Chemicals (Cont'd.)

Chemical	Extracting Solvent	GC Detector	Reference
Dichlorvos	Hexane	P-FPD	Lamoreaux and Newland, 1978
DDT	Acetone, ether, benzene	^{63}Ni-ECD	Lichtenstein *et al.*, 1977
Fenitrothion	Hexane	^{63}Ni-ECD, FID, P-FPD	Maguire and Hale, 1980
Diclofop-methyl	Methanol, benzene, acetonitrile	^{63}Ni-ECD	Martens, 1978; Smith, 1979a
Butachlor, CDAA	Methylene chloride	Radioactive detector	Marvel *et al.*, 1978
Methoxyclor	Benzene	^{63}Ni-ECD, FID	Mathur and Morley, 1975
Endosulfan	Hexane	^{63}Ni-ECD	Miles and Moy, 1979
2,4,5-Trichlorobiphenyl, 2,2',4,4',6-pentachlorobiphenyl	Methanol	^{63}Ni-ECD, FID	Moza *et al.*, 1979
Benzo[a]pyrene, monolinuron, dieldrin	Hexane, ether	^{63}Ni-ECD, thermionic N-FID	Muller and Korte, 1975
Pentachloronitrobenzene	Acetone, methanol	^{63}Ni-ECD	Murthy and Kaufman, 1978
Toxaphene	Hexane, acetone, methanol	^{63}Ni-ECD	Nash *et al.*, 1977
Maneb, zineb, ETU	Hexane, benzene, methanol	^{63}Ni-ECD	Nash and Beall, 1980
N-nitrosodipropyl-, diethyl-, dimethylamine, pendimethalin	Methylene chloride	FID	Oliver *et al.*, 1979
Dichlorononane	Diethyl ether	FID	Omori and Alexander, 1978
Bentazon	Methanol, ethyl acetate	S-FPD[d]	Otto *et al.*, 1977
Malathion, malaoxon	Hexane, acetone	^{63}Ni-ECD	Paschal and Neville, 1976
Organophosphates	Ethyl acetate	FID, P-FPD	Rosenberg and Alexander, 1979
2,4,5-T, 2,4,5-TCP	Ethyl acetate	FID	Rosenberg and Alexander, 1980a,b

Table IV-1. Gas Chromatography Assay of Chemicals (Cont'd.)

Chemical	Extracting Solvent	GC Detector	Reference
Glyphosate	NH_4OH	FID	Rueppel et al., 1977
Trifluralin, Tri-allate	Acetonitrile	^{63}Ni-ECD	Smith, 1979b
Polybutadiene	Diethyl ether	FID	Tsuchii et al., 1978
Phosphamidon	Acetonitrile	P-FPD	Varty and Yule, 1976
Simazine, atrazine, Brometryne, linuron	Methanol	Thermionic N-FID	Walker, 1978; Walker, 1976a,b
Trifluralin	Benzene, ethyl acetate	^{3}H-ECD	Wheeler et al., 1979
n-Alkylamines	HF, NaOH	FID	Wszolek and Alexander, 1979
2,4,5-T, 2,4-D, picloram	Ether, chloroform, hexane	^{3}H-ECD	Yoshida and Castro, 1975
Propyzamide	Ethyl acetate	^{3}H-ECD	Zandvoort et al., 1979

[a] N-P-FID; Nitrogen-phosphorus flame ionization detector

[b] ECD; Electron capture detector

[c] P-FPD; Phosphorus flame photometric detector

[d] S-FPD; Sulfur flame photometric detector

wants to obtain (both OECD and EPA recommend labeling in more than one position). For example, if one is interested in the cleavage of the acetate moiety from the aromatic ring of 2,4,5-T (2,4,5-trichlorophenoxyacetate) and in the fate of acetate moiety in soil, the acetate moiety should be labeled with ^{14}C (Koch, 1975). On the other hand, if one is interested in the degradation pathway of the aromatic rings, the rings will be labeled with ^{14}C (Rosenberg and Alexander, 1980a,b). The use of ^{14}C-labeled chemicals in soil studies has been divided into three sections: (i) Assay for loss of radioactivity of the test chemical; (ii) Identification of metabolic intermediates; and (iii) $^{14}CO_2$ evolution. Although no new pertinent analytical procedures are included in (i) and (ii), these sections are summarized (with recent references) to indicate the general techniques used.

(i) Assay for the Loss of Radioactivity of Test Chemicals

Accuracy in the determination of the loss of radioactivities of test chemicals from soil samples largely depends on the extraction and clean-up. These procedures have been previously discussed (see Section III.B.1.a). To separate the parent ^{14}C test chemical from its metabolites, TLC is usually employed. Then the spots are scraped and placed in a scintillation fluid for determination of radioactivity (Martens, 1978) or the radioactivity may be determined by a radioscanner (Martens, 1978; Rhodes, 1980). In the case of pure culture studies, the total radioactivity in the culture fluid is determined by directly transferring a portion of culture fluid to a scintillation vial and counting. Radioassay techniques are also applied in studies of the leaching of chemicals in soils and binding to soil components (Berard and Rainey, 1979; Katan et al., 1976; Lichtenstein et al., 1977).

The determination of total radioactivity remaining in the soil samples (residue ^{14}C-labeled parent compound plus any nonvolatile ^{14}C-labeled degradation products) can be done by two techniques. In both procedures it is necessary to first remove any nonbiologically adsorbed $^{14}CO_2$, and then to convert the ^{14}C-labeled organic fraction to small molecules, e.g. $^{14}CO_2$, which can be trapped and quantitated by liquid scintillation counting. One method utilizes pyrolysis (combustion) of the soil sample (Goswami and Green, 1975; Hsu and Bartha, 1976; Otto et al., 1977; Rhodes, 1980; Wheeler et al., 1979) in which the sample is oxidized and then trapped by scintillation counting. The other method involves release of the nonbiological $^{14}CO_2$ fraction by acidification followed by wet oxidation of the organic fraction and trapping of the resulting $^{14}CO_2$ (Atlas and Hubbard, 1974; Bancroft et al., 1979).

(ii) Identification of Metabolic Intermediates

The accuracy of results relies on the extraction and cleanup. The ^{14}C-labeled products and the ^{14}C-labeled parent compound are generally separated by TLC. Standards of the probable metabolites of the test chemical (when they are known) are chromatographed with the unknown. The radioactive spots on plates are located by means of autoradiography (Rosenberg and Alexander, 1980a; Spillner et al., 1979; Venkateswarlu and Sethunathan, 1979) or by use of a radioscanner (Rhodes, 1980). By comparison of the Rf values of the unknown metabolic products with the standards, the possible identity of the unknown metabolites may be obtained. With the assistance of autoradiography or radioscanning, the portions of unknown ^{14}C-labeled metabolites are detected, each portion is collected, extracted and crystallized. When large enough amounts of the unknown are available, the unknown can be identified with analysis by IR, NMR, mass spectroscopy, etc. Besides TLC, GC has been used for separating ^{14}C-compounds or ^{14}C-metabolites (Nelson and Zeikus, 1974). Additionally, HPLC equipped with a radioactive flow detector can be used for ^{14}C-product separation and identification.

(iii) $^{14}CO_2$ Evolution

Although the disappearance of the radioactivity of ^{14}C-labeled test chemicals in soil samples is a good indication of degradation, the detection of $^{14}CO_2$ evolution provides a definite indication of the mineralization of the chemical, since water and CO_2 are the final biodegradation products. Soil samples treated with a ^{14}C-labeled test chemical are generally placed in a glass container and incubated under aerobic or anaerobic conditions. In aerobic studies, the headspace is generally purged with CO_2-free air into a gas-trapping solution in scintillation fluid. The containers may be attached in series to a manifold apparatus. The gas-trapping solution can also be placed in a sidearm of the glass container, and the solution changed periodically (Bartha and Pramer, 1965). For the studies of $^{14}CO_2$ evolution under submerged soils, or inert gas, evolved $^{14}CO_2$ is periodically withdrawn by purging the headspace with nitrogen (Murthy and Kaufman, 1978) or hydrogen (Marinucci and Bartha, 1979b) or by removal with a gas-tight syringe followed by analysis by GLC (Hackett et al., 1977). Alternatively, $^{14}CO_2$ from flooded soils or soils purged with inert gases is collected in small vials suspended about the soil (Tiedje, 1977; Tiedje and Mason, 1974; Venkateswarlu and Sethunathan, 1979). The $^{14}CO_2$ is trapped in solutions such as ethanolamine, hyamine hydroxide, KOH or NaOH solution (0.1 N or 1N). The radioactivity of $^{14}CO_2$ in the trapping solution is determined by transferring a known volume of the trapping solution to a scintillation solution and counting in a scintillation counter (i.e., Bartha and Pramer, 1965; Marinucci and Bartha, 1979a,b; Tiedje, 1977; Tiedje and Mason, 1974) or mixing the trapping solution with the scintillation fluid prior to purging. If a KOH or NaOH solution is used for trapping CO_2, the radioactivity of $^{14}CO_2$ can also be determined by converting it to $Ba^{14}CO_3$ and its radioactivity is then counted (Betts et al., 1976; Ou et al., 1978; Sielicki et al., 1978). The $^{14}CO_2$ is verified by acidifying and counting (Ou et al., 1978). If $^{14}CO_2$ is the only ^{14}C-material present, then no radioactivity should remain.

Two basic types of incubation systems have been developed and utilized to measure the rate and extent of biodegradation of ^{14}C-labeled chemicals; the open system and the closed system.

(a) Open System for $^{14}CO_2$ Evolution

The open system employs a manifold assembly. The advantages of this system are in maintaining the desired atmospheric composition, multiple trapping assemblies, and precision. Disadvantages include its space requirements, personal attention required, and its complexity when applied to large multiflask experiments. Variations of this basic approach have been used for the biodegradation of, for example, $^{14}C(U)$-2,4-D (Ou et al., 1978), $^{14}CF_3$-labeled trifluralin (Wheeler et al., 1979), ^{14}C-carboxyl-labeled 2,4,5-T (Byast and Hance, 1975), ^{14}C-labeled pesticides (Figure IV-2, Goswami and Koch, 1976), and using nitrogen gas, for the anaerobic biodegradation of pentachloronitrobenzene (Figure IV-3, Murthy and Kaufman, 1978). The $^{14}CO_2$ evolved from the soil is trapped in a suitable trapping solution as previously mentioned.

(b) Closed System for $^{14}CO_2$ Evolution

The closed system utilizes static or intermittent aeration. An example is the biometer flask (Bartha and Pramer, 1965; Figure IV-4). The system requires less equipment, space, and personal attention; however, the oxygen content is not controlled and reduction can affect metabolic processes (Goswami and Koch, 1976). Examples of this system include studies on the biodegradation of ^{14}C-p-nitrophenol (Wahid et al., 1980), metabolism of $^{14}C(U)$-carbofuran (Venkateswarlu and Sethunathan, 1979), $^{14}C(U)$-2,4,5-T in various tropical soils (Rosenberg and Alexander, 1980b), ^{14}C-lignocelluloses (Crawford and Crawford, 1976), $^{14}C(U)$-1,2,3- and 1,2,4-trichlorobenzene (Marinucci and Bartha, 1979a,b), and ^{14}C-triallate and ^{14}C-diallate (Anderson and Domsch, 1976; 1980).

(c) Distinction Between Volatile ^{14}C-material and $^{14}CO_2$

A feature common to almost all the aforementioned systems is that unambiguous distinction between $^{14}CO_2$ and other volatile ^{14}C activity trapped in the base requires separate special analysis. Various systems have been developed to deal with this problem. Anderson and Domsch (1980) fitted Erlenmeyer flasks with ground-glass joint reflux columns containing from bottom to top: a trap for the volatilized pesticide (glass-wool plug coated with paraffin), a trap for $^{14}CO_2$ (soda lime on glass wool), and a top layer of soda lime to protect the $^{14}CO_2$ trap from becoming saturated with atmospheric CO_2. These same investigators trapped ^{14}C-diallate in toluene and paraffin oil traps and $^{14}CO_2$ was trapped in methanol/ethanolamine solution (Anderson and Domsch, 1976). Helweg (1979) similarly used paraffin to trap volatile ^{14}C-material from ^{14}C-2AB. Marinucci and Bartha (1979a,b) flushed the incubation vessel with negative pressure to first trap ^{14}C-material ($^{14}C(U)$-1,2,3-and 1,2,4-trichlorobenzene) in toluene or xylene-based cocktail and then $^{14}CO_2$ in phenethylamine fluor. The vials were connected in series (Figure IV-5). The system used teflon, stainless steel, and glass to reduce the affinity of the system for the trichlorobenzenes. Ethylene glycol (Ebing and Schuphan, 1979; Richey et al., 1977; Wheeler et al., 1979) and activated charcoal (Oliver et al., 1979) have been used to trap volatile ^{14}C-material. Additionally, polyurethane plugs have been used to trap ^{14}C-material (Kearney and Kontson, 1976; Marvel et al.,

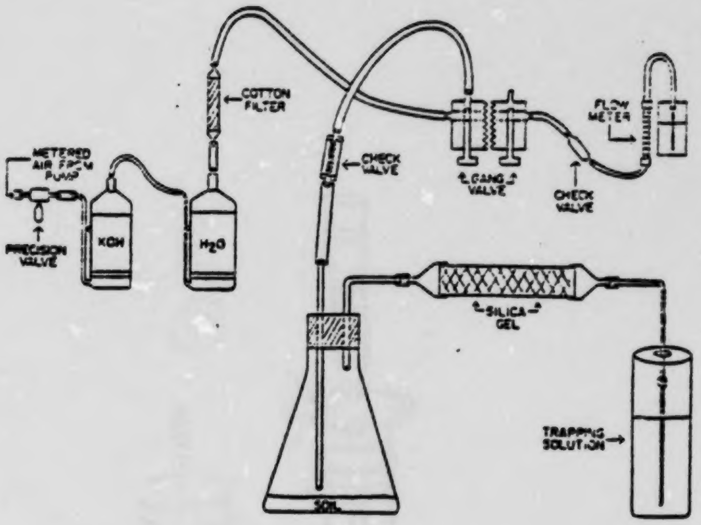
METERED AIR FROM PUMP
PRECISION VALVE
KOH
H_2O
COTTON FILTER
CHECK VALVE
GANG VALVE
CHECK VALVE
FLOW METER
SILICA GEL
SOIL
TRAPPING SOLUTION

Figure IV-2. Apparatus for trapping $^{14}CO_2$ from degradation of ^{14}C-labeled pesticides (Goswami and Koch, 1976).

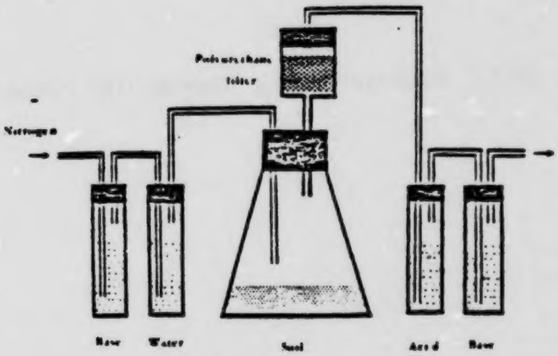
Polyurethane filter
Nitrogen
Base
Water
Soil
Acid
Base

Figure IV-3. Apparatus used to study the degradation of ^{14}C-labeled pesticides in an anaerobic environment (Murthy and Kaufman, 1978).

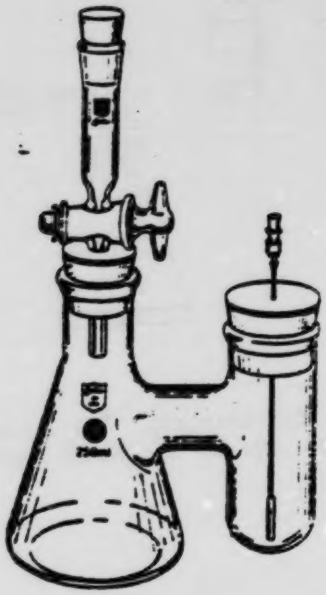

Figure IV-4. Biometer flask (Bartha and Pramer, 1965).

1978; Murthy and Kaufman, 1978; Nash et al., 1977; Rueppel et al., 1977). The system developed by Marvel et al. also contained ascarite to trap $^{14}CO_2$. The apparatus consisted of a 250 ml Erlenmeyer flask and a two-piece trapping tower (Figure IV-6). A variation of this system was developed for studying anaerobic biodegradation of ^{14}C-labeled compounds (Rueppel et al., 1977). Anaerobiosis was achieved and maintained with nitrogen gas.

e. GC-MS Techniques

Although the gas chromatograph is an effective instrument for the separation and quantification of degradation products, it most often does not provide positive identification of these degradation products. For this reason, gas chromatography (GC) has been combined with mass spectrometry (MS). The extraction procedures and derivatization techniques are the same as used with gas chromatography alone. In general, GC/MS techniques involve first enumerating GC conditions, then injecting the sample into the GC/MS, and having the chemicals enter the MS after appropriate separation in the GC. In the MS, the organic molecules in a vacuum are changed into charged particles and fragmented in a variety of ways. The resulting fragmentation pattern can then be used to identify the products. Metabolites of 2,4,5-T (Rosenberg and Alexander, 1980a), Mirex (Carlson et al., 1976), and Gossyplure, a sex pheromone (Henson, 1977), for example, have been identified by GC/MS.

f. O_2 Consumption

Although no new analytical procedures were found in the literature, it is worth noting a modification of a standard technique. Oxygen consumption is most often used as an indication of aerobic heterotrophic microbial activity on a chemical and can be used with CO_2 evolution and disappearance of the test chemical to provide definitive evidence of biodegradation.

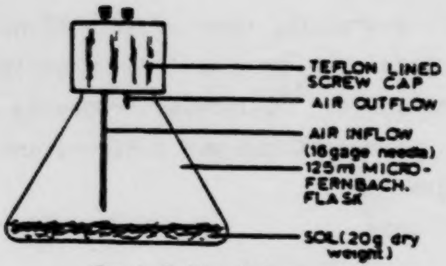
TEFLON LINED SCREW CAP
AIR OUTFLOW
AIR INFLOW (16 gage needle)
125 ml MICRO-FERNBACH FLASK
SOIL (20g dry weight)

FIG. 1. *Soil incubation vessel.*

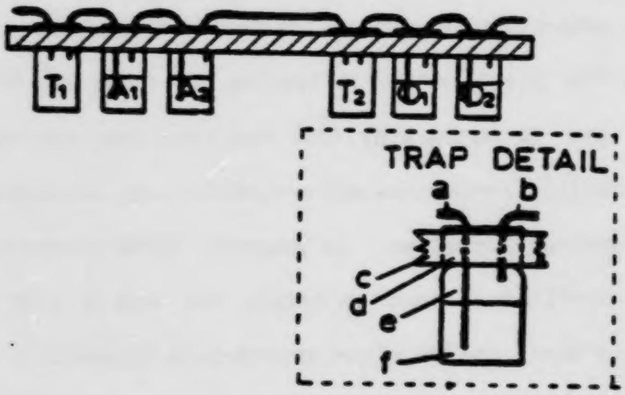
T1
A1
A2
T2
O1
O2
TRAP DETAIL
a
b
c
d
e
f

Figure IV-5. Radioactive trichlorobenzene and CO_2 trapping system. T_1 and T_2 are backflow traps. A_1 and A_2 contain a toluene or xylene-based fluor for trichlorobenzene trapping. O_1 and O_2 contain a phenethylamine fluor for CO_2 trapping. Trap detail: (a) inflow (18-gauge stainless steel); (b) outflow (18-gauge stainless steel); (c) 0.625-in. (ca. 1.6-cm) wood mounting board; (d) epoxy-cemented scintillation vial cap (24 mm); (e) glass scintillation vial; (f) trapping fluor (Reprinted from Marinucci and Bartha, 1979. Apparatus for monitoring the mineralization of volatile ^{14}C labeled compounds. Appl. Environ. Microbiol., 38:1020-1022, with permission of the American Society for Microbiology.)

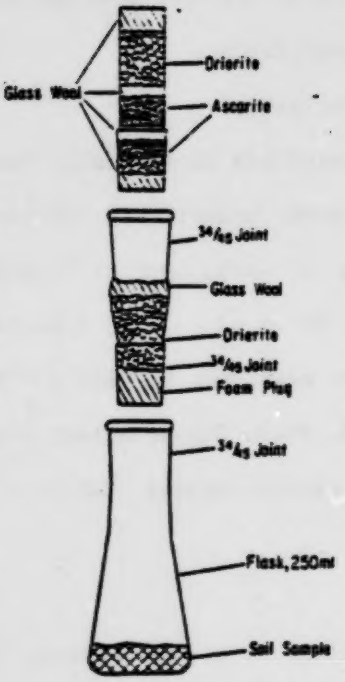
Drierite
Glass Wool
Ascarite
34/45 Joint
Glass Wool
Drierite
34/45 Joint
Foam Plug
34/45 Joint
Flask, 250ml
Soil Sample

Figure IV-6. Aerobic soil metabolism incubation system (Marvel et al., 1978).

Oxygen consumption can be measured with an oxygen electrode and BOD dilution bottles or Warburg and Gilson respirometers. If the test chemical is ^{14}C-labeled, then initial and final $^{14}CO_2$ can be measured and radiorespirometry done (Bancroft _et al_., 1979). Endogenous and treated controls are prepared to determine the extent of oxidation of the test chemical and if O_2 consumption is due to uncoupled oxidative phosphorylation.

g. CO_2 Evolution

The technique is basically the same as described for $^{14}CO_2$ evolution (trap CO_2 with base) except that the base must be titrated with acid normally in the presence of $BaCl_2$ and an indicator such as phenolphthalein (Congregado _et al_., 1979; Ou _et al_., 1978; Sielicki _et al_., 1978). Two tests using this technique were mentioned by OECD (1979b). One test uses a static system (Kibble, R.A., 1966, Ph.D. Dissertation, University of Liverpool) and the other uses a continuous aerated system (Quilt, P., 1972, Ph.D. Dissertation, University of Bath).

2. Bioassays

Determinations of the persistence in soil of pesticides, including insecticides and herbicides, often use bioassays. This is especially true with field tests. The method can show the persistence of a pesticide residue toxic to sensitive animals or plants in soil. Furthermore, bioassays are desirable because they do not rely on extraction techniques and are relatively inexpensive because they do not require the development of an analytical procedure. A disadvantage of the method is that pesticides may form complexes with soil or firmly bind to soil and become nontoxic to sensitive animals or plants, even though the same concentration in aqueous solution would be very toxic.

a. Plant Bioassays for Herbicides

The bioassays for the determination of the persistence of herbicides in soils are based on the growth inhibition of sensitive plants. The degree of growth inhibition is generally correlated with the concentration of herbicide in soil as long as the concentration of the herbicide is not too high or low. The parameters used for determination of a herbicide's persistence and toxicity in soil are lengths of roots and shoots (Moza *et al.*, 1979; Jacques and Harvey, 1979; Zandvoort *et al.*, 1979), total dry weight of the plant (Sharom and Stephenson, 1976), and percentage of the plants that emerge and develop normally (Dibble and Bartha, 1979c). Some of the plants assayed and chemicals tested include ^{14}C-trichlorobiphenyl compounds with carrots and sugarbeets (Moza *et al.*, 1979), a kerosene spill in a wheat field (Dibble and Bartha, 1979c), dinitroaniline herbicides and oat seeds (Jacques and Harvey, 1979), gherkin and flax studied with propyzamide (Zandvoort *et al.*, 1979), and field studies with cucumbers and metribuzin (Sharom and Stephenson, 1976).

For field bioassays, soil plots receive various concentrations of herbicide. Seeds are then planted at various intervals. For laboratory and greenhouse bioassays, soil samples to be tested are generally placed in pots or wooden boxes, treated with the herbicide, and then the seeds are planted.

b. Insect Bioassays for Insecticides

The terms "persistent" and "non-persistent" are often applied to the longevity of a chemical in the environment. However, in soil, at least, the persistence of a chemical can be affected by binding to soil particles. Thus, a "non-persistent" compound may only be nonpersistent because it forms nonextractable, bound residues that can be potentially toxic to organisms. One method used to determine toxicity employs insects. Lichtenstein *et al.* (1977) exposed fruit flies (*Drosophila melanogaster*, Meigen) to aliquots of soils previously treated with 10 ppm of ^{14}C-methylparathion or ^{14}C-fonofos and extracted with benzene, methanol, and acetone. These extracted soils containing the ^{14}C-bound residues of each compound were placed in bioassay jars and 50 flies added. Mortality counts were performed at intervals over a 72-hour exposure period. Controls showed no insect mortality over the 72-hour period.

C. Evaluation of Soil Biodegradation Techniques

1. Factors Affecting Degradation

The key factors that affect the degradation of synthetic chemicals in soil appear to be soil type, depth of soil, test chemical concentration, soil microorganisms and acclimation, physical environment (including pH, temperature, O_2 availability, redox potential, moisture content of the soil, and sorption), and additional carbon sources. Optimizing these factors in laboratory studies in general maximizes microbial activity and thus biodegradation of the chemical.

Representative studies which deal with the influence of these factors on degradation of chemical compounds in soil are described below. However, a large number of studies in the literature have determined persistence of a chemical as a function of changes in the environmental conditions, but have not distinguished between degradation and loss due to photolysis, volatilization, and minor alteration in the molecule. The last section (B.1.d.iii) indicates the importance of monitoring volatilization of the test chemical.

a. Soil Type

A very wide spectrum of soil types has been used in studies of the degradation of synthetic chemicals in the soil environment. The soil types vary from sand (low in organic matter content, usually less than 1 percent) to muck soil or peaty soil (high in organic matter content, usually higher than 50 percent). Although it is known that soil type does influence the degradation of synthetic chemicals in soil, it is less certain whether the rate of degradation correlates with organic matter content of the soil. Considering the fact that microbial activity is associated with organic matter content, the soil types that have higher organic matter content should give higher degradation rates. However, the presence of organic matter in soil also affects the movement, adsorption and vaporization of the chemical (e.g., Katan et al., 1976; Sprankle et al., 1975), and results in immobilization of free-state enzymes. Such effects may reduce the chances of cells or enzymes finding their substrates and thus perhaps reduce the degradation rates. Rosenberg and Alexander (1980a,b) studied the degradation of 2,4,5-T in four tropical clay soils and found that the rate and extent of degradation was correlated with the organic matter content of the soils (Table IV-2). Fournier (1975) studied the degradation of ^{14}C-isoproturon in four soil types and found that the most rapid and extensive degradation of ^{14}C-isoproturon occurred in calciferous soil. Lande et al. (1979), using carboxyl ^{14}C-acrylamide, demonstrated dissimilar half-lives (Time for appearance of one-half the evolved $^{14}CO_2$) in four soil types (Figure IV-7).

b. Soil Depth

With an increase of soil depth, there is generally a decrease in oxygen tension, volatilization, and loss of the test chemical. A change in the types and abundance of microorganisms also accompanies a change in soil depth. Torstensson and Hammarstrom (1979) studied the degradation of trichloroacetic acid (TCA) at various depths in soils. The relative degradation capacity expressed as time for degradation to 50 percent in different levels of the studied soil profiles is summarized in Table IV-3. They found that the time for 50 percent degradation of TCA ranged from 2 weeks in the 0-5 cm level of the Funbo-Lovsta soil to 9 weeks or more in the lower levels of the Galbo, Lovsta-Lot

Table IV-2. Organic Matter Content, pH, and Degradation of $^{14}C(U)$-2,4,5-T in Four Tropical Soils Incubated for 120 days (Rosenberg and Alexander, 1980a,b)

Soil	Organic Matter (%)	pH	% Disappearance[a]	$^{14}CO_2$ Evolved[b]
Philippine Maahas Clay (PH)	3.0	6.8	40	34
Trinidad Silty Clay (TR)	2.8	6.1	23	21
Nigeria Silty Clay (NI)	2.4	5.7	15	11
Puerto Rico Nipe Clay (PR)	2.2	5.8	5	5

[a] Initial concentration was 10 ppm. Values are maximal disappearance.

[b] Percent ^{14}C evolved as $^{14}CO_2$. Values are maximal $^{14}CO_2$ evolution.

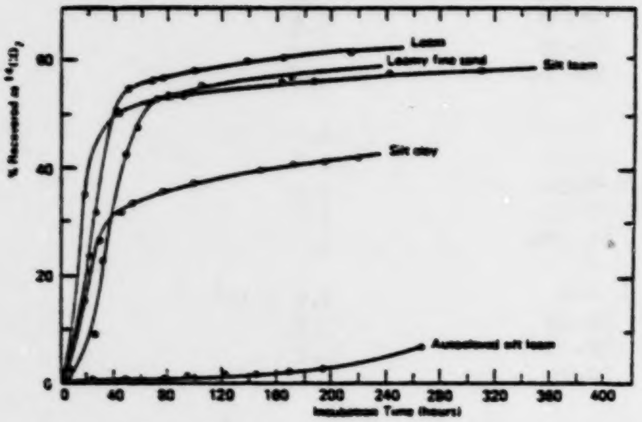

Figure IV-7. Carbon-fourteen carbon dioxide production from ^{14}C-carboxyl acrylamide (25 ppm) in soils incubated with aerobic conditions at ambient temperature (22°C) (Lande et al., 1979).

Table IV-3. Relative Degradation Capacity for TCA in Soil Samples from Different Levels of Soil at Five Localities (Torstensson and Hammarstrom, 1979)

Locality	Sampling level (cm)	Growth of microorganisms on TCA-agar (number/g soil)	Time for degradation to 50% (days)
Funbo-Lovsta	0-5	3.1 x 10^5	10
	20-25	1.2	16
	45-50	0.9	16
	70-75	1.4	16
	95-100	1.8	16
Galbo	0-5	4.1 x 10^5	21
	20-25	0.5	47
	45-50	1.5	66
	70-75	3.1	59
	95-100	13	68
Lovsta-Lot	0-5	4.9 x 10^5	18
	20-25	2.3	47
	45-50	0.2	57
	70-75	0.1	61
Tuvattnet 1	0-5	39 x 10^6	26
	20-25	116	51
	45-50	3.0	>64
	70-75	3.8	>64
Tuvattnet 2	0-5	50 x 10^6	30
	20-25	20	55
	45-50	17	56
	70-75	20	55
	95-100	8.0	54

and Tuvattnet 1 soils. Tate (1979) found a similar correlation of soil depth with degradation of salicylate, acetone, and succinate.

c. Test Chemical Concentration

The persistence of synthetic chemicals in soils is often affected by the dose applied. At higher concentrations, the chemical often persists longer than at lower concentrations. At lower concentrations, however, either the rate of degradation may be directly proportional to the chemical's concentration or little or no biodegradation below a threshold value will occur. The relationship between concentration and rate is predicted by Michaelis-Menten kinetics. In silt loam soil, the half-life at 22°C of 500 ppm carboxyl ^{14}C-acrylamide was 94.5 hours, compared to 18 hours at 25 ppm (Lande *et al.*, 1979). Byast and Hance (1975) noted that the rate of $^{14}CO_2$ evolution was greater from ^{14}C-carboxyl-labeled 2,4,5-T at 1 ppm than at 15 ppm. However, the experiments were run consecutively and not simultaneously, so experimental conditions could have varied. Ou *et al.* (1978) found that the rate of degradation of $^{14}C(U)$-2,4-D (evolution of $^{14}CO_2$) was indirectly related to the concentration above 500 ppm (Figure IV-8). This relationship was unaffected by the type of soil or the type of 2,4-D used (technical grade, 97 percent pure and formulated, 41 percent 2,4-D as the dimethylamine salt). Both Byast and Hance and Ou determined their rates by measuring $^{14}CO_2$ evolution. In both cases, however, actual rate values were not given. Dibble and Bartha (1979a,c) found that smaller more frequent applications of oil sludge to soil yielded higher overall degradation rates than infrequent application of large batches. Tiedje and Mason (1974) reported that increased concentrations of nitrilotriacetate (NTA) (10-600 ppm) increased the rates of degradation. Ahmad *et al.* (1979a) reported no significant alteration of the half-lives of phorate, terbufos, and their oxidized products due to higher or lower application rates under similar field conditions. Although the maximum

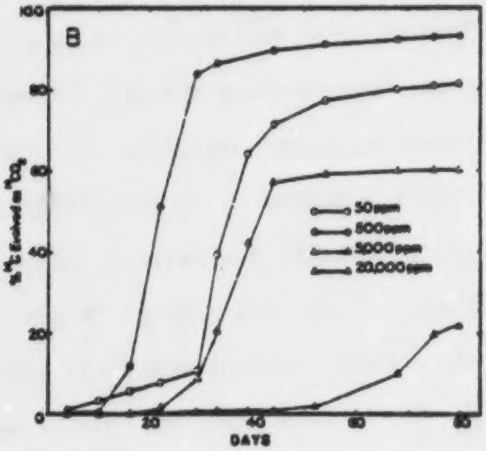

Figure IV-8. Percent $^{14}CO_2$ evolved from Webster soil (silty clay loam) treated with formulated 2,4-D (Ou *et al.*, 1978).

percent of $^{14}C(U)$-1,2,4-trichlorobenzene (TCB) converted to $^{14}CO_2$ per day occurred between 10 and 25 ppm and then decreased with increased 1,2,4-TCB concentration (to 100 ppm), the absolute rate of its degradation (nmoles/day) reached its maximum between 10 and 25 ppm and remained constant thereafter (Marinucci and Bartha, 1979a).

d. Soil Microorganisms and Acclimation

Organic chemicals in soil are broken down largely by microorganisms. Soils differ in the types and abundance of microorganisms they contain and this affects the persistence of chemicals in soil. Degradation of organic chemicals in different soil types has been discussed previously (c.1.a). Although degradation rates vary considerably from one soil type to another, the variations cannot be attributed exclusively to the microbiological composition of the soil, since in complex environments such as soil, other soil and climatic factors such as temperature, moisture content, acidity, organic matter, etc. may be expected to influence degradation.

Acclimation also affects the capacity of soil microbes to activate chemical compounds. Evidence for acclimation of soil microorganisms to a test chemical has been obtained by a number of researchers. For example, Helweg (1979) showed enhanced degradation of 2-aminobenzimidazole in a soil perfusion system inoculated with liquid or soil from the soil perfusion apparatus which had been pretreated with the chemical. Loos et al. (1979) found a direct relationship between the concentration of 2,4-D- and 4-chloro-2-methyl-phenoxyacetate (MCPA)-degrading microorganisms in soil samples and the amount of compound degraded. Trichloroacetic acid (TCA) was found to degrade with no lag period with further additions of the chemical (McGrath, 1976) as were phenoxyacetate and 2,4-D (Rosenberg and Alexander, 1980b) (Figure IV-9).

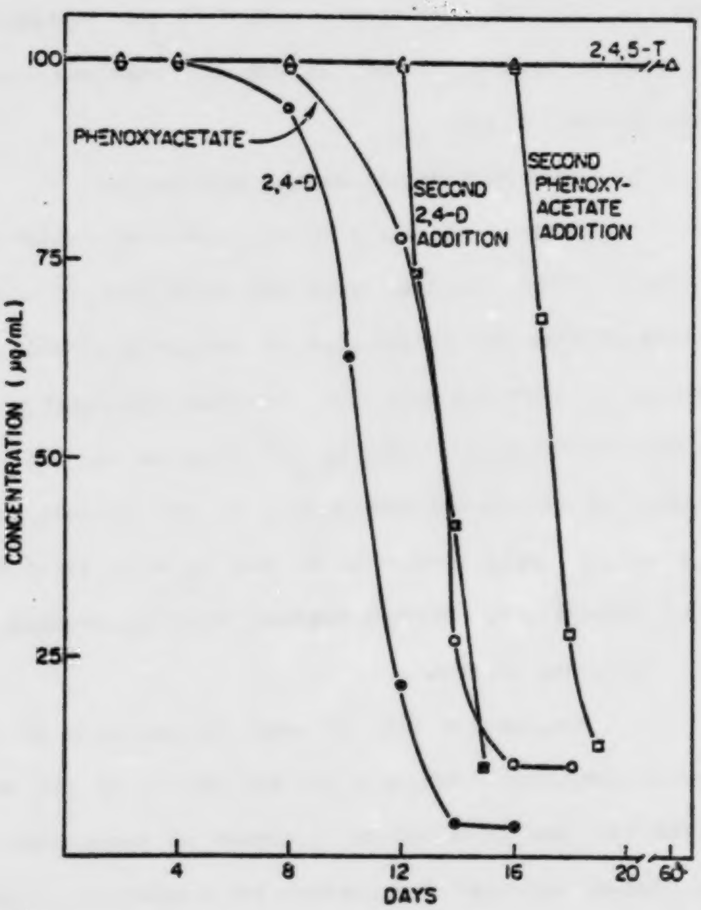

Figure IV-9. Disappearance of 2,4-D and phenoxyacetate from suspensions of Maahas clay amended with 100 µg/ml of each compound (Rosenberg and Alexander, 1980b).

Rajaram and Sethunathan (1976) showed an increase in the rate of hydrolysis of parathion in flooded soils after the addition of a parathion-hydrolyzing Pseudomonas sp. Parathion degradation was enhanced in soils when treated with an acclimated mixed culture grown on parathion as the sole carbon source (Daughton and Hsieh, 1977).

e. Physical Environment - pH, Temperature, Oxygen Availability, Redox Potential, Moisture Content of the Soils, and Absorption

The hydrogen ion concentration of the soil or of the degradation medium can affect the rate at which both chemical and microbiological decomposition occur. For biodegradation, the pH effect is generally very specific, depending on the particular chemical and the particular microbe involved. Hance (1979) found that linuron, propyzamide, and dichlorprop were degraded in soil much more rapidly at pH values approximating neutrality. Dibble and Bartha (1979a) adjusted the pH of soil with $CaCO_3$ from 5.0 to 7.8 and found maximal oil sludge degradation at the highest pH (Figure IV-10).

The influence of temperature on the breakdown of chemicals in soil is a complex relationship. Increase in temperature generally results in increased rates of volatilization, desorption, and leaching. Microbial activity in the soil is also directly influenced by temperature. Generally, at temperatures below 10°C, the overall transformation of a chemical is slower than at temperatures above 20°C. Helweg (1979) noted that $^{14}CO_2$ evolution from ^{14}C-labeled-2-aminobenzimidazole was exponentially related to the inverse of the absolute temperature in accordance with the Arrnenius equation. Dibble and Bartha (1979a) found that although increasing the temperature from 5 to 37°C increased overall CO_2 evolution from oil sludge there was no increase in biodegradation (activity) rate above 20°C. The half-lives of simazine and linuron increased from 29 to 209 days and 36 to 106 days, respectively,

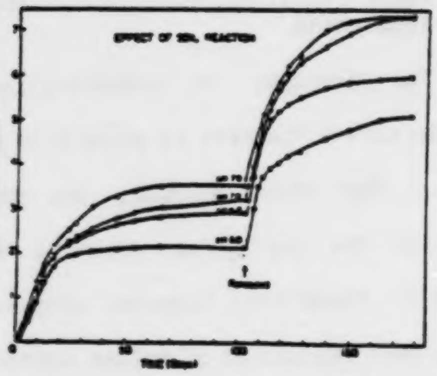

Figure IV-10. Effect of soil reaction (pH) on carbon dioxide evolution from oil sludge (Reprinted from Dibble and Bartha, 1979. Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol., 37:729-739, with permission of the American Society for Microbiology.)

from 30 to 5°C (Walker, 1976b). Tiedje (1977) demonstrated that increasing the incubation temperature from 10 to 50°C increased the rate of degradation of ^{14}C-carboxyl-EDTA in soil (Figure IV-11). The rate of degradation was initially slow at 50°C, but accelerated after 9 weeks suggesting catabolic enzymes were initially inactive, but later a thermotolerant population was selected which catalyzed EDTA degradation (Tiedje, 1977). In silt loam soil, the half-lives of ^{14}C-acrylamide at 10, 22, and 37°C were 96, 18, and 9.6 hours, respectively (Lande _et_ _al_., 1979).

The time of year at which the soil is collected can affect the biodegradation of a chemical. ^{14}C-Acrylamide degradation (measured as $^{14}CO_2$ evolution) in a silt loam soil taken as the ground thawed in March, had a half-life about 2.5 times that of the half-life for acrylamide degradation in the same soil type collected in June (Lande _et_ _al_., 1979). Tiedje (1977) reported opposite results for ethylenediaminetetracetate (EDTA) incubated in loamy fine sand. Thus, a winter sample metabolized EDTA two times faster than the same soil collected in summer.

Most degradative reactions catalyzed by soil microbial communities require the presence of oxygen. Although, in general, no new pertinent data were found since the 1975 report, three papers are worth mentioning. Marinucci and Bartha (1979a) found that continuous flushing with hydrogen gas or alternate weekly flushing of soil with air and hydrogen gas depressed $^{14}C(U)$-1,2,3- and 1,2,4-trichlorobenzene mineralization. Tiedje (1977) reported no $^{14}CO_2$ from ^{14}C-carboxyl-EDTA under anaerobic conditions (10 percent hydrogen, 90 percent nitrogen) while aerobic conditions or subsequently aerating the anaerobic system yielded from 5 to 15 percent $^{14}CO_2$ (Figure IV-12). Lande _et_ _al_. (1979) recovered more ^{14}C-acrylamide from anaerobically-incubated soil than from

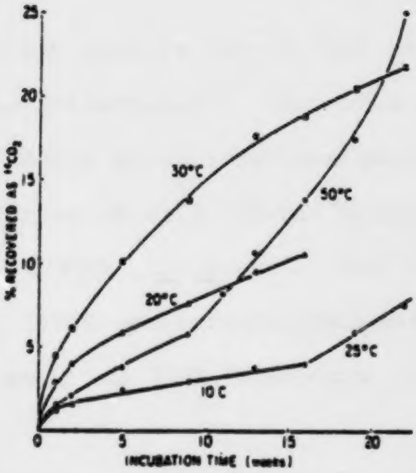

Figure IV-11. Effect of incubation temperature on biodegradation of ^{14}C-carboxyl-EDTA (2.8 ppm) in Spinks soil. The flasks at 10°C were shifted to 25°C at 16 weeks (Tiedje, 1977).

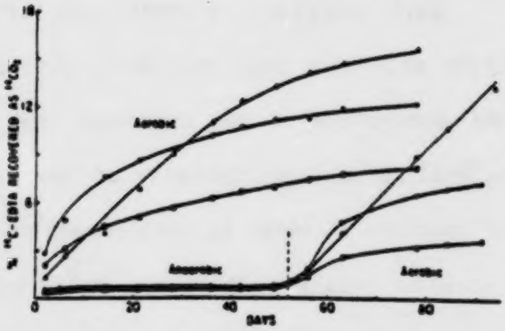

Figure IV-12. Biodegradation of ^{14}C-carboxyl-EDTA (4.4 ppm) under aerobic and anaerobic-to-aerobic conditions in sediments from the Detroit River-Lake Erie area: Livingstone Channel [○], Trenton Channel [△], and East Outer Channel [□] (Tiedje, 1977).

aerobically-incubated soil, suggesting a longer lifetime under anaerobic conditions.

Willis et al. (1974) have pointed out that factors other than mere exclusion of oxygen (e.g., redox potential) may determine the rate of pesticide degradation in anaerobic environments. These researchers investigated the relationship between the oxidation reduction potential (Eh) and the rate of degradation of the herbicide trifluralin. It was found that the rate of degradation of trifluralin was much more rapid under more reducing conditions (Figure IV-13).

Soil moisture content can influence persistence of a chemical directly by affecting soil microbial activity or indirectly by influencing the initial absorption of the chemical, the rate at which the chemical diffuses into the soil or the availability of the absorbed chemical (Gerstl et al., 1979a). The presence of water in soil caused an increase in the degradation of simazine and linuran (Walker, 1976a,b), dinitroaniline herbicides (Jacques and Harvey, 1979), and parathion (Gerstl et al., 1979b) in soils treated with these chemicals. The soil water content greatly influenced the evolution of $^{14}CO_2$ from ^{14}C-labeled 2-aminobenzimidazole (2-AB) (Figure IV-14). $^{14}CO_2$ evolution at a water content corresponding to 28 to 94 percent of field capacity (5 to 17 percent total water content of the soil) was exponentially related to $^{14}CO_2$ evolution with increasing water content (Helweg, 1979). The increase in activity with soil moisture is probably due to an increase in microbial activity which leads to an increase in degradation of the chemical.

Knowledge of the movement of pesticides and other potential pollutants through soil is necessary for evaluation of their environmental fate. The degree to which a chemical is adsorbed by the soil will directly affect its mobility (Sprankle et al., 1975). The sorption of certain organic chemicals to

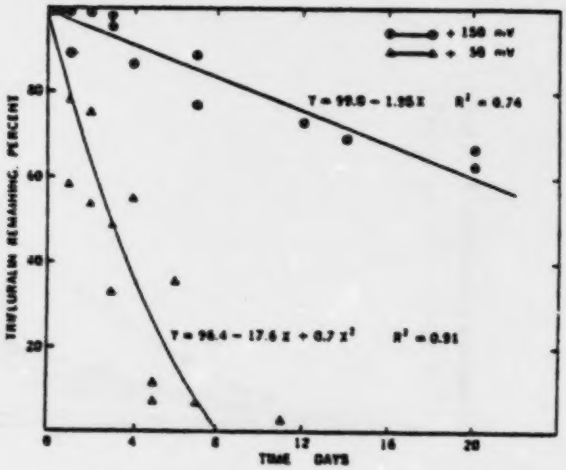

Figure IV-13. Disappearance of trifluralin from soil suspensions as a function of redox potential and time. The curve $Y = 99.0 - 1.95X$ was developed from soil suspensions at redox potentials at +450, +250, and +150 mV. The curve $Y = 96.4 - 17.6X + 0.7X^2$ was developed from soil suspensions at redox potentials at +50, 0, and -50 mV (Willis _et al_., 1974).

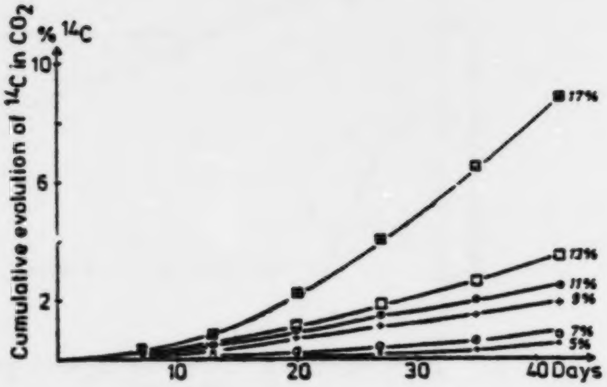

Figure IV-14. Cumulative evolution of ^{14}C in CO_2 from soil supplemented with 4 ppm ^{14}C-labeled 2-AB and incubated at 25°C with 5, 7, 11, 13, and 17% water (Helweg, 1979).

soil particles strongly influences their biodegradation by microorganisms (Helling, 1976; Daughton et al., 1979c). Thus, the cationic herbicides, diquat and paraquat, appear to be completely protected from degradation when sorbed to montmorillonite (Burns and Audus, 1970). Wszolek and Alexander (1979) reported a correlation between resistance to microbial attack of n-alkylamines bound to clay and their molecular weights. However, these investigators found also that microorganisms may facilitate removal of the sorbed substrate from clay by production of extracellular enzymes or a pH change. A relationship between pH and sorption was noted for the degradation of glyphosate in soil (Moshier and Penner, 1978; Sprankle et al., 1975). Sorption of glyphosate was also related to the phosphate level, formation of colloidal Fe and Al precipitates, and soil type (Moshier and Penner, 1978; Sprankle et al., 1975). Presumably, these factors enhanced microbial activity and thus degradation.

f. External Carbon Source

The role of soil organic matter in degradation of organic chemicals has been discussed in an earlier section (C.1.a). Several researchers in their degradation studies have modified soils with the addition of external carbon sources which differ in type and quantity from the organic matter naturally occurring in soils. This section was adequately reviewed in the 1975 report and with one exception, no new pertinent data were found in the literature. Dimock (1975) found that a soil pseudomonad readily attacked 2,4-D as the sole carbon source but attacked 2,4,5-T and 2-(2,4,5-trichlorophenoxy)-propionic acid (2,4,5-TP) only in the presence of a readily degradable organic substrate (i.e., sodium benzoate). Since no difference in the cell yield occurred when the bacterium was grown on benzoate and either 2,4,5-T or 2,4,5-TP or on benzoate alone, it was concluded that the herbicides were degraded cometabolically.

Additionally, it was shown that peptone and beef extract directly stimulated cell metabolism of the herbicides while benzoate simply increased cell yield.

2. General Discussion of Various Test Methods

Unlike natural waters, soils are generally richer in microbial content and have, therefore, been used extensively as a microbial inoculum in degradation studies without amendment or added microorganisms. A test method utilizing microorganisms occurring in natural soil approaches more closely the conditions encountered in nature. The use of soil may eliminate the need for adjustment of various biodegradation parameters (although these parameters still should be monitored) such as microbial population and diversity, nutrient and oxygen concentration, in an attempt to simulate the natural soil environment. The use of soil in a biodegradation test in the laboratory permits the investigator to perform degradation studies under controlled conditions; e.g., where losses due to volatilization, leaching, etc. can be controlled. However, soil, when used in the laboratory, is somewhat removed from many naturally occurring environmental conditions. For example, the moisture content in the laboratory degradation study is usually maintained relatively constant; while under natural conditions, the moisture content will change substantially. The soil from different geographic locations will vary considerably in terms of physicochemical properties and microbial composition which may introduce serious variations in the results of degradation studies of different laboratories. Furthermore, the presence of a complex medium such as soil will often introduce additional steps in extraction and cleanup. Also, the presence of soil may preclude the assay of biodegradation by many analytical procedures. For example, measurement of oxygen consumption with natural soil as the biological material has generally not been used for studying degradation; the reason being the high endogenous rates of soil respiration which may make the interpretation of oxygen uptake data more difficult.

In light of the complex nature of soil and the various methods to determine biodegradation of chemicals in soils, it would be advantageous to develop a standard set of procedures and techniques to study metabolic transformation of potential pollutants in soil. Before discussing the test methods examined in this report, various methods being evaluated by OECD and the U.S. EPA will be discussed.

Before soil can be used in degradation experiments, it must be collected, treated, and analyzed. Soils should be collected to a depth of 20 cm from an appropriate number of different sites within a plot (Pramer and Bartha, 1972). The time of year should also be considered since the biochemical activities of soil tend to be greater during late spring to early fall than during winter and early spring (Lande _et_ _al_., 1979) although not true in all cases (Tiedje, 1977). To insure homogeneous material, composite samples should be mixed thoroughly and sieved to 1-5 mm in diameter. The U.S. EPA (1975; 1978a) suggests similar treatment in its recommendations of acceptable procedures for conducting soil metabolism (see Table IV-4 for the characteristics of these procedures). OECD (1979a) outlined a method for determining particle size of a soil (~2 mm was recommended). If possible, soil samples should be used immediately, but may be stored in thin-walled polyethylene bags at 15-20°C for no more than 30 days. Storage at subfreezing temperatures has been shown to decrease the microbial population and microbial activity in soil (Pramer and Bartha, 1972). The ability of a soil to pass through sieves is a good index of its moisture status. The water content of soil is important since drying of soil can cause drastic qualitative and quantitative population shifts and alter enzymatic

Table IV-4. Characteristics of Some Acceptable Procedures for Conducting Soil Metabolism Studies (U.S. EPA, 1975, 1978)

Test	Soil Moisture (%)	Drying Method	Sieved Particle Size (mm)	Culture Vessel	Duration (days)	Analysis
Aerobic Metabolism						
Audus (1960)	60-80	air	--	Erlenmeyer flask	< 100	Substrate disappearance
Bartha and Pramer (1965)	70	air	1-2	Biometer flask	20	CO_2, $^{14}CO_2$
Betts *et al*. (1976)	80	air	2	Biometer flask	60	$^{14}CO_2$
Cornfield (1961)	75	air	2	Test tube	60	CO_2
Fleeker *et al*. (1974)	50-75	air	--	Erlenmeyer flask, Biometer flask	20	Metabolite $^{14}CO_2$
Gingerich and Zimdahl (1976)	80	air	0.83	Glass jar	210	Substrate disappearance
Kazano *et al*. (1972)	80	air	2	Biometer flask	32	$^{14}CO_2$
Messersmith *et al*. (1971)	60-80	air	1-2	Biometer flask	300	$^{14}CO_2$
Parr and Smith (1969) Parr *et al*. (1970)	70	air, oven	0-2	Manifold assembly (bottle, Erlenmeyer flask)	< 100	Substrate disappearance
Schooley *et al*. (1975)	80	air	--	Mason jar	60	$^{14}CO_2$
Smith *et al*. (1973)	75	air	--	Biometer flask, Erlenmeyer flask)	244	Substrate disappearance $^{14}CO_2$

Table IV-4. Characteristics of Some Acceptable Procedures for Conducting Soil Metabolism Studies (U.S. EPA, 1975, 1978) (Cont'd.)

Test	Soil Moisture (%)	Drying Method	Sieved Particle Size (mm)	Culture Vessel	Duration (days)	Analysis
Anaerobic Metabolism (flooded soil)						
Betts et al. (1976)	--	air	2	Biometer flask	< 100	$^{14}CO_2$
Gingerich and Zimdahl (1976)	--	air	0.83	Glass jar	40	Substrate disappearance
Gowda and Sethunathan (1976)	--	air	--	Test tube	55	^{14}C-Endrin disappearance
Parr and Smith (1969)	--	air, oven	0-2	Manifold assembly (bottle, Erlenmeyer flask)	< 100	Substrate disappearance
Parr et al. (1970)						
Anaerobic Metabolism (inert gas; N_2Ar, CO_2)						
Parr and Smith (1969)	--	air, oven	0-2	Bottle, Erlenmeyer flask	< 100	Substrate disappearance
Parr et al. (1970)						

activity and thus, the rate and/or pathway of degradation of the test chemical (Walker, 1976a,b). The soil is normally air dried; however, oven drying on a separate sample from the one to be used for testing is normally used to determine water-holding capacity. Generally, the soil is moistened to 50-80% of its water-holding capacity (U.S. EPA, 1978a recommends 75%). OECD (1979a) discusses a method to estimate the maximum water-holding capacity of an oven-dried sample. Finally, since soils are so variable, certain parameters should be routinely characterized. These include: pH, Eh, organic matter, particle-size distribution, and cation exchange capacity (OECD, 1978, 1979a).

After the soil has been analyzed and treated, it is ready for use. Under laboratory conditions, the soil is placed in incubation vessels appropriate for the experimental objectives. Pramer and Bartha (1972) suggest a ratio of 10 cc of air for each 1.0 g of soil to maintain adequate available oxygen in a closed system. If the ratio is less than 10:1 (as suggested by U.S. EPA, 1975), then daily aeration is needed to prevent anaerobiosis. However, if anaerobic studies are conducted then the soil should be either flooded to 2-4 cm above the soil or purged with an inert gas(es) such as N_2 or Ar, or both methods should be used (Table IV-4; U.S. EPA, 1975; OECD, 1978).

Since the objective of many laboratory studies is to determine biodegradation, many investigators have attempted to demonstrate that the loss of the test chemical was due to biological and not physico-chemical reactions. The simplest procedure is to compare persistence of the test chemical in sterilized and unsterilized portions of a soil sample. However, since any sterilization process may affect abiotic reactions in soil, comparisons between sterile and non-sterile soils do not provide conclusive evidence that the process is biodegradation (see Section IV.A. on sterilization). OECD (1978) and U.S. EPA (1978a) recommend irradiation or intermittent sterilization as mentioned above.

Substances to be tested for biodegradability are best dissolved in the water used to moisten the soil with the soil then added to permit moistening from below by capillary action (OECD, 1978). If a test chemical must be dissolved in solvent, the amount of solvent added to the culture vessel should be as small as possible and allowed to evaporate to diminish any possible toxicity to the microbial population. Water is added first, then the soil. The soil should be mixed to insure further uniform distribution of the test chemical. The maximum non-toxic test chemical concentration to the soil microflora is also determined (OECD, 1978).

Although the incubation temperature may be a function of the experimental objectives, U.S. EPA (1975, 1978a) recommends 18 to 30°C, while OECD (1979a) recommends 25 ± 2°C. U.S. EPA (1978a) recommends sampling at 0-7 days, 2 and 3 weeks, and 1-12 months while OECD (1979a) recommends sampling at 0, 4, 8, 16, 32, 64, 128 days or until the patterns of degradation are established. The exact analytical methods used (HPLC, GC, MS, TLC, $^{14}CO_2$ evolution, etc.) to determine degradation and/or metabolites will depend on the test chemical, extraction procedures, and experimental objectives (U.S. EPA, 1975, 1978a). U.S. EPA (1975, 1978a) recommends data collecting until 90% loss of the chemical occurs and recommends identifying residues comprising more than 10% of initial application or 0.01 ppm, whichever is greater.

In an effort to obtain a complete understanding of the environmental fate and persistence of a particular chemical, numerous laboratory test methods are used. These include static- as well as shaking-flask tests as described in Section A.1.a. If the test chemical has broad uses, tests with three or more different soils are preferred (U.S. EPA, 1978a).

In a number of laboratory degradation test methods, soil is suspended in water, and therefore, one deals with an aqueous suspension of soil rather than soil at moisture levels generally encountered under natural conditions. When sizable amounts of soil are used with the water, the system simulates flooded soil or sediment conditions. However, in many cases, soil is used as an inoculum in the test procedure and not as a medium for degradation and, therefore, the quantity of soil suspended in the aqueous medium is very small. This might limit the availability of many undefined nutrients present in soil; such nutrients may be essential for certain soil microorganisms to proliferate under the test conditions and degrade the test chemical. The advantage of the method is that in cases where a dilute soil suspension is used, certain analytical measurements, e.g., disappearance of U.V. absorption, can be made directly on the sample or on the supernate obtained after centrifugation. The extraction and cleanup steps in such cases may be minimal. Furthermore, when the soil suspended in aqueous solution is used in degradation studies, a more uniform distribution of the test chemical may be obtained. This may permit a uniform exposure of the test chemical to the entire microbial population, as well as removal of relatively homogeneous samples for degradation analysis.

Soil perfusion systems are another method to study biodegradation of the test chemical. Soil perfusion systems permit a constant exposure of microorganisms to air, water and the test chemical and subsequently the degradation environment is perhaps more potent than occurring under natural conditions. Additionally, the system is an excellent tool for enriching microorganisms which will degrade the test chemical. Soil perfusion systems have generally been used to study aerobic degradation; the simulation of flooded and anaerobic soil conditions has not been attempted with this test method. U.S. EPA (1975) has proposed the use of a soil perfusion system as a means to study degradation of test compounds.

Since the degradation and identity of metabolites of test chemicals in soil present difficulties, the fate of these chemicals has been frequently determined with pure or mixed culture and cell-free extract studies (see Sections A.1.b. and A.1.c.). Although pure cultures allow for simple and well-defined conditions and thus easier assessment of degradation and metabolite identification than soil, they are unsuitable for preliminary screening for biodegradability since organism(s) which can metabolize a compound in pure culture are not necessarily those responsible for its degradation in the soil. Thus, results from pure culture studies may be impossible to extrapolate to the natural conditions. Degradation by pure cultures of microorganisms will also fail to allow interaction among natural environmental conditions as well as interaction with other microorganisms. Since certain biochemical reactions are catalyzed by extracellular enzymes, these enzymes may degrade organic compounds in soil. If pure cultures of microorganisms are used in degradation studies, the extracellular soil enzymes produced by other microorganisms will be absent. The advantages of using mixed soil culture are discussed in Section A.1.b.ii.).

The origin of pure culture of microorganisms for degradation work have varied considerably. Among the techniques used to isolate pure cultures of microorganisms for studying degradation of chemicals, the enrichment-culture technique is the most widely used (see Section A.1.b.i.).

Degradation studies utilizing cell-free extracts have been used by researchers to study the enzymatic mechanisms of degradation. Since the membrane permeability barrier is absent when a compound is exposed to the cell-free extract of a microorganism, one can always question if the compound will be degraded by the intact cell and, if so, will the rates be comparable to those observed with the cell-free extracts and can the data be useful in predicting the fate of the compound in natural communities.

Model ecosystems used by researchers to date are oriented more towards answering questions concerning bioaccumulation of the chemical compounds than environmental persistence or biodegradation. Most of these systems are not well defined in terms of their microbial composition and therefore, their reproducibility and subsequently their usefulness in environmental persistence studies is uncertain. However, the metabolic fate in the food chain is quite reproducible in these systems. In the ecosystem designed by Nash et al. (1977), the fate of fungicides and insecticides was studied in a sandy loam soil. Although useful, especially with radiolabeled compounds, interpretation of the data may be limited since the mechanisms observed in these systems may not always be the same as under natural conditions.

Laboratory studies on the biodegradability of chemicals are an important part in determining the fate and persistence of the chemicals in the environment and thus their potential exposure. However, many laboratory studies deduce patterns and pathways of degradation as well as metabolites under rigorously controlled conditions, and the question arises whether these data can be justly and accurately extrapolated to the "real world."

Thus, in an effort to obtain a more complete study of the degradation of a particular chemical, many investigators have studied biodegradation in field experiments as well as in laboratory experiments. Such parameters as water content of the soil, temperature, light, pH, nutrient concentration, inoculum size, chemical concentration, oxygen availability, and prior exposure to the test chemical continuously vary making degradation rate studies difficult. In addition, loss of the compound due to biodegradation as compared to leaching, volatilization, percolation, or photodecomposition, may not be easily distinguished.

A paper reviewed in the 1975 report indicated that differences in the disappearance curves of eight phosphate insecticides occurred between laboratory and field experiments. The differences were attributed to the lack of strict control of several environmental parameters in the field experiment.

Other studies since 1975 have employed both laboratory and field experiments. Banks et al. (1979) found fluridone degradation in Miller clay and Lufkin fine sandy loam to be more rapid in the field than under controlled conditions. However, the loss of fluridone from sterilized and nonsterilized soils was insignificantly different indicating abiotic degradation and making data interpretation difficult. In contrast, trichloroacetic acid (TCA) disappeared faster under laboratory conditions than in field studies regardless of the soil type or whether the soil was untreated or pretreated with TCA (McGrath, 1976). The degradation of 2,4,5-T in a clay soil approximated first-order kinetics in the laboratory, but in three out of five field experiments the degradation pattern did not fit; the model overestimated the rates of loss due to incorrect soil moisture measurements (Walker and Smith, 1979). Smith (1979a) determined that the degradation of diclofop-methyl under field conditions appeared to parallel that in the laboratory with diclofop-acid as the main breakdown product and 4-(2,4-dichlorophenoxy)phenol as a minor metabolite. However, the laboratory and field experiments were not conducted simultaneously.

Thus, laboratory data, although allowing for more precise determination of metabolites, degradation pathways and kinetics, and abiotic and biotic disappearance of the test chemical, may not accurately reflect the fate of that chemical in the environment. Therefore, laboratory studies might be more productive if they are compared to information established in field experiments.

The use of radiolabeled compounds as an analytical method and the trapping of $^{14}CO_2$ and ^{14}C materials in open and closed incubation systems is discussed in Section B.1.d. Two systems (developed by Marvel et al., 1978 and Anderson and Domsch, 1980) utilize relatively simple equipment, little space, and permit free exchange of air between the incubation flask and the atmosphere. However, since neither system was tested with highly volatile organic compounds, it is not certain whether the polyurethane or glass-wool plugs would be effective in trapping these compounds. Both EPA and OECD recommend the biometer flask system and, if needed, an open system. In light of the previous discussion on incubation systems (see Sections B.1.d.iii.(a),(b),(c)), a modification of the trapping tower-flask setup would be more practical for consideration.

D. Cost Analysis for Testing Biodegradability of Chemicals in Soil

Degradation studies can be carried out in three phases: (i) preliminary or screening tests to determine the overall biodegradability of a compound, (ii) identification of metabolites and establishment of degradation pathways, and (iii) biodegradation under simulated ecological conditions. The analytical method for preliminary testing could be a direct measurement of the parent compound and/or the metabolite, or an indirect method; such as microbial growth, oxygen consumption, or CO_2 evolution. The costs associated with the analytical procedures applicable to degradation studies in the soil are based on a 7.5-year life for small equipment (e.g., shakers, spectrophotometer, oxygen meter, etc.) and a 15-year life for capital equipment (e.g., scintillation counter, gas chromatograph, etc.), the number of days to perform the tests (including extraction difficulties with soil), the educational and experience level of the worker(s), the overhead, and the type of laboratory performing the test. A detailed degradation study may be supplemented with terrestrial ecosystem and/or pure culture studies. The cost for each preliminary test is calculated for one and for twenty compounds (Table IV-5).

Table IV-5. Cost Analysis for Preliminary Biodegradability Test in Soil Environment

Biodegradation Test Method	Analytical Method	Equipment Needed and Their Cost	Equipment Cost for the Test	Chemicals, Glassware, and Misc. Supplies	Labor/Cost $100/day	Overhead 125% of Profes-sional Services	Total Cost	Cost/Compd.
Soil incubated with test chemical under stationary conditions (1)	Gas chromatography	Gas chromatograph, $12,000; Centrifuge, $6000	$ 50	$115	$ 500	$ 625	$1290	$1290
" (20)	"	"	300	1200	3000	3750	8250	415
Soil incubated with test chemical under stationary conditions (1)	Radioactive material (measuring loss of parent material)	Scintillation counter, 18,000; Centrifuge, 6000	60	1350 (incl. labelled material)	500	625	2535	125
" (20)	"	"	400	18000 (incl. labelled material)	3400	4250	26050	1300
Soil incubated with test chemical under stationary conditions (1)	Radioactive material (measure evolution of $^{14}CO_2$; e.g., in a biometer flask)	Scintillation counter, 18,000;	30	1450 (incl. labelled material)	300	375	2155	2155
" (20)	"	"	200	19500 (incl. labelled material, biometer flasks)	2000	2500	24200	1210
Soil incubated with test chemical under stationary conditions (1)	Bioassay	-	-	105	300	375	780	780
" (20)	"	-	-	750	2000	2500	5250	265

Table IV-5. Cost Analysis for Preliminary Biodegradability Test in Soil Environment (Cont'd)

Biodegradation Test Method	Analytical Method	Equipment Needed and Their Cost	Equipment Cost for the Test	Chemicals, Glassware, and Misc. Supplies	Labor/Cost $100/day	Overhead 125% of Professional Services	Total Cost	Cost/Compd.
Soil suspended in aqueous solution (1)	Gas chromatography	Gas chromatograph, $12,000; Centrifuge, $6000	$ 50	$110	$ 500	$ 625	$1285	$1285
" (20)	"	"	250	1200	2300	2875	6625	330
Soil suspended in aqueous solution (1)	Radioactive material (measure loss of parent compound)	Scintillation counter, 18,000; Centrifuge, 6000	60	1100 (incl. labelled material)	500	625	2285	2285
" (20)	"	"	370	18000 (incl. labelled material)	3000	3750	25120	1255
Soil suspended in aqueous solution (1)	Radioactive material (measure evolution of $^{14}CO_2$)	Scintillation counter, 18,000	40	1200 (incl. labelled material, biometer flask or gas train assembly)	400	500	2140	2140
" (20)	"	"	200	19500 (incl. labelled material, biometer flask or gas train assembly)	2000	2500	24200	1210
Soil suspended in aqueous solution (1)	U.V. Absorption	Spectrophotometer, 4000; Centrifuge, 2000	15	75	200	250	450	450
" (20)	"	"	80	450	900	1125	2555	125

Table IV-5. Cost Analysis for Preliminary Biodegradability Test in Soil Environment (Cont'd)

Biodegradation Test Method	Analytical Method	Equipment Needed and Their Cost	Equipment Cost for the Test	Chemicals, Glassware, and Misc. Supplies	Labor/Cost $100/day	Overhead 125% of Professional Services	Total Cost	Cost/Compd.
Soil suspended in aqueous solution (1)	Colorimetric (e.g., chloride ion release)	Spectrophotometer, $2000; Centrifuge, $6000	$ 25	$110	$ 300	$ 375	$ 810	$ 810
" (20)	"	"	120	600	1300	1625	3645	180
Soil perfusion test (1)	Colorimetric (e.g., chloride release)	Spectrophotometer, 2000; Centrifuge, 6000	55	150	700	875	1780	1780
" (20)	"	"	400	1200	5000	6250	12850	645
Soil perfusion test (1)	Gas chromatography	Gas chromatograph, 12,000; Centrifuge, 6000	100	150	1000	1250	2500	2500
" (20)	"	"	600	1800	6000	7500	15900	795

(The number in parenthesis indicates the number of compounds tested.)

The results of the preliminary screening will be helpful in deciding the necessity and approach to be used in more intensive studies. If the preliminary test indicates low biodegradability, the intensive study might use an enrichment culture technique to determine if any breakdown can be detected. If moderate or high degradation is noted then the intensive study may concentrate on metabolite identification.

Once the biodegradability of a compound has been established, detailed studies can be undertaken to elucidate biochemical pathways and characterize metabolites. For this purpose, more well-defined systems such as pure cultures, resting-cell suspensions, and cell-free extracts can be used. However, the results thus obtained may be of little ecological or environmental relevance. This phase of the biodegradation test is time consuming and expensive and, therefore, should be undertaken only with those compounds for which preliminary evidence for the presence and accumulation of metabolites has been obtained. Custom synthesis of radiolabeled compounds and suspected metabolites needed to identify unknown metabolites of the test chemical will be expensive. The cost estimate for an intensive study of biodegradation is as follows:

One Compound

Labor	-	Ph.D. Level (20K), six months	$10,000
Overhead	-	125%	12,500
Equipment	-	GC-MS (have analysis performed by a commercial laboratory), Scintillation counter (cost for six months ≃ $750)	4,000
Chemicals, Glassware, and Misc. Supplies	-	Materials for thin-layer and paper chromatography, autoradiography, etc.	1,100
TOTAL COST	-		$27,600

The U.S. EPA has reviewed the economic impact incurred to meet EPA guidelines for registering pesticides in this country (U.S. EPA, 1978b, 43 FR 39644). They estimated a cost of $29,000 per test for the aerobic soil metabolism studies and $31,900 per test for the anaerobic soil metabolism studies.

Twenty Compounds

Labor	-	Ph.D. Level (20K), five years	$100,000
Overhead	-	125%	125,000
Equipment	-	GC-MS (cost $150,000, depreciate over 10 years; cost for 5 years, $75,000), Scintillation counter, cost for 5 years, $4,500	75,000
Chemicals, Glassware, and Misc. Supplies	-		10,000
TOTAL COST	-		$310,000
COST/COMPOUND			$ 15,500

The labor cost assumes a detailed study of four compounds per year.

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V. PHOTOCHEMICAL AND CHEMICAL ALTERATIONS

A. Degradation of Chemicals in the Atmospheric Environment

1. Introduction

Atmospheric transport is a major route for the distribution of chemical contaminants of the environment (Risebrough et al., 1968; Risebrough, 1969; Stanley et al., 1971; Compton et al., 1972; Wood, 1974). In addition, for many of the prominent air pollutants (e.g., SO_x, NO_x, CO, hydrocarbons, particulate matter, photochemical smog, etc.), the atmospheric route provides the predominant route of exposure to man and other biota. Thus, an understanding of the atmospheric chemistry of these contaminants is extremely important to considerations of their persistence and fate in the environment. Unlike the alterations discussed in other sections of this report, atmospheric alterations of chemical contaminants are entirely non-biological.

Substances emitted to the atmosphere, as to other media, may remain unchanged or be converted to more or less objectionable products. The variety of physical and chemical influences on a chemical contaminant is extremely complex, and reviews of the reactions and mechanisms of the assimilation process are presented elsewhere (Finlayson and Pitts, 1976; Rowland, 1976, 1977; Haagen-Smit and Wayne, 1968; Altshuller and Bufalini, 1971). Presented here will be only a brief discussion of the unique conditions to which a contaminant is exposed in the atmospheric environment and the experimental procedures that are used to study a chemical's reactions in the atmosphere.

The atmospheric media provides an excellent matrix for photochemical or oxidative alteration of chemical contaminants. The intensity and wavelength (>290 nm in the lower atmosphere) of sunlight are capable of initiating a variety of reactions. For example, the energy provided by sunlight is able to break carbon-carbon and carbon-hydrogen bonds, cause the photodissociation of nitrogen dioxide to nitric oxide and atomic oxygen, and photolytically excite a relatively large number of oxygen molecules due to their high concentration. This relatively high concentration of oxygen (20.9% V/V) makes it one of the most important participants in various reactions with air pollutants since the rates of reactions are concentration dependent (Haagen-Smit and Wayne, 1968). Similar reasoning can be used for reactions with water vapor (0.1 to 5% V/V) and carbon dioxide (.03 - 0.1% V/V). Examples of the type of reactions that occur in the atmospheric environment are as follows:

$$NO_2 + h\nu \xrightarrow{< 430\ nm} NO + O(^3P)$$

$$O + O_2 \longrightarrow O_3$$

$$NO + O_3 \longrightarrow NO_2 + O_2$$

$$OH + CO \longrightarrow CO_2 + H$$

$$H + O_2 \longrightarrow HO_2$$

$$HO_2 + NO \longrightarrow OH + NO_2$$

Conditions in the upper atmosphere are even more vigorous. Above heights of 50 miles, oxygen exists almost exclusively in the atomic form. Between 10 and 20 miles above the earth a region of high ozone concentration exists. Above this level the energy of the incident sunlight is much higher (< 290 nm) because the ozone level is not available to absorb the high energy light. It is generally believed that, through turbulence and diffusion, contaminants from the lower atmosphere will be exposed to the upper atmosphere and this may be a major sink for environmental contaminants.

Physical processes may also be important to both fate and the residence time of chemicals in the atmosphere. Adsorption of substances on particulate matter and subsequent gravitational settling appear to provide a major source of pesticide (and presumably other chemicals) removal from the air

(Risebrough et al., 1968). A "washout" mechanism caused by falling drops of rain may also be important, especially for water soluble materials (Bufalini et al., 1979). In addition, the adsorption of chemicals on particulate matter may drastically affect the photochemical reaction rate (Klein and Korte, 1971).

Thus, the atmospheric conditions that need to be simulated in the laboratory for the evaluation of a chemical's persistence and fate are extremely complex. A variety of approaches have been utilized.

2. Techniques Used for Determining Atmospheric Degradation

Most studies of atmospheric chemistry have concentrated on processes which have physiological or toxicological significance. These have included processes important to smog formation, eye irritation, oxidant levels, etc. For example, the reactivity of hydrocarbons are often studied in relation to the rate of oxidation of nitric oxide to nitrogen dioxide, an important first step that results in photochemical smog formation. Rarely is a chemical species studied just to determine its chemical reactions in the atmosphere. The interpretation of results varies considerably, depending upon whether one is evaluating long-term (e.g., trace environmental contamination) or short-term phenomena (e.g., smog formation). For example, reactive hydrocarbons in the atmosphere are considered extremely undesirable because of their contribution to the formation of smog. This is somewhat analagous to dissolved oxygen depletion in water by high BOD compounds. The techniques which are discussed in this report are considered mostly for their ability to assess a chemical's persistence and fate rather than to predict air pollution consequences.

a. Long-Path Infrared Cells

Long-path infrared cells have frequently been used to study the atmospheric photooxidation of hydrocarbons and nitric oxide. Stephens and coworkers (Hanst et al., 1956; Stephens, 1958) were among the first to use long-path infrared spectroscopy (LPIR) in the study of air pollution chemistry. The apparatus they used consisted of a 3 meter, 18 inch diameter stainless steel tube which contained a three mirror multiple-reflection system. The total path-length was 216 meters (72 passes). The cell was connected to a single beam infrared spectrometer.

Tuesday and Glasson (Tuesday, 1963; Glasson and Tuesday, 1970a, 1970b, 1970c, 1971) used a similar 3 meter stainless steel long-path cell, but with only 40 passes (total path-length 120 meters). Irradiation was supplied by a number of eight-foot black light fluorescent bulbs (F96 - 8/BL) mounted inside the long-path cell. The cell was attached to a modified Perkin-Elmer Model 21 Infrared Spectrometer. A full description of the apparatus is given by Tuesday (1961).

The experimental procedure consisted of first determining the actinic light intensity of the lamps by measuring the rate of nitrogen dioxide photodissociation at very low concentrations in nitrogen gas. Because the quantum yield is close to unity over the spectral region of interest, this is a reproducible and common method of determining the light intensity in order to compare it to the intensity of sunlight (Tuesday, 1961). The appropriate equation is:

$$-d[NO_2]/dt = K_d[NO_2]$$

where $K_d = \Phi K_a$. K_a is the rate of light absorption and Φ is the primary quantum yield.

The long-path cell was evacuated to a pressure of less than 10μ Hg, and known pressures of nitric oxide and nitrogen dioxide were expanded into the cell from an attached glass vacuum system. Hydrocarbons with high vapor pressures at room temperature were also expanded into the cell from the glass vacuum system while hydrocarbons with low vapor pressures were added by a 10-μl syringe to an electrically heated stainless steel manifold containing several high vacuum values and a silicone rubber septum. The hydrocarbon was expanded from the manifold into the cell and the manifold was flushed with nitrogen. Nitrogen was then added to the long-path cell to about 600 mm Hg. After the addition of oxygen (155 mm Hg), the final pressure was brought to 760 mm Hg with a small amount of nitrogen. The concentrations of hydrocarbons in most cases varied from 0.25 to 5.0 ppm, with initial concentrations of nitric oxide and nitrogen dioxide of 0.38 ppm and 0.02 ppm, respectively. Quite often these concentrations were varied when the investigators were studying the effect of nitric oxide concentration on hydrocarbon photooxidation or vice versa. These concentrations are well within those existing in polluted atmospheres [3 to 5 ppm hydrocarbon expressed as ppm carbon and 0.2 to 0.6 ppm nitrogen oxides (Korth et al., 1964)]. The amount of thermal oxidation of nitric oxide in the cell prior to irradiation was less than 1% at the concentrations used (Glasson and Tuesday, 1970a). The concentrations of nitrogen oxides, hydrocarbons, and photochemical products were measured by scanning the infrared spectra.

A more recent study used LPIR for an assessment of the effects of fluorocarbons on the phenomena of photochemical smog (Japar et al., 1974). These researchers used two (reference and sample) standard long path-length cells which were internally coated with FEP (fluorinated ethylene-propylene) Teflon in order to decrease any effects due to wall reactions. The cell optics were adjusted to obtain a total path length of 40 meters. Irradiation was supplied by an external 17.5" arc-length, 1200 watt Hanovia medium pressure mercury arc which was surrounded by a double-walled quartz jacket cooled with distilled water. The light passed through six 3" x 3" irradiation ports placed along the length of the cell. The port consisted of 1/4" thick glass which passed only wavelengths longer than 310 nm. The actinic intensity of the lamp measured as nitrogen dioxide dissociation in nitrogen was 0.131 min^{-1}. This intensity was noted to be comparable to a Los Angeles noontime measurement of 0.37 min^{-1}.

The reaction mixtures were expanded into the LPIR cells from an attached glass vacuum system. Concentrations of fluorocarbons, nitrogen dioxide, and olefin varied from 5 to 8 ppm, 10.5 to 12.5 ppm, and 15 to 34 ppm, respectively. The LPIR cells were pressured to slightly less than 1 atmosphere with compressed air which was passed through a Matheson 450 Gas Purifier and a 3" diameter, 2.5' Dierite column to remove any oil and most of the water present in the air. Concentrations of the fluorocarbon, olefin, and nitrogen dioxide were measured by scanning the infrared spectra before, during, and after irradiation through sodium chloride windows in the LPIR cells.

Modern analytical methods typically rely upon Fourier Transform (FT)-IR techniques to monitor reagent and product concentrations. FT methods are much more sensitive than more conventional continuous wave techniques and allow faster data acquisition. Gay et al. (1976) utilized the chamber illustrated in Figure V-1 to study the oxidation of chlorinated ethylenes. The chamber consists of six connected borosilicate pipes, which are separated by aluminum spacers with Teflon gaskets, and has an internal volume of 690 liters. Each chamber section is illuminated by eight GE-F40-BLB black light lamps with a maximum output at 366 nm. The chamber contains an eight mirror optical system and serves as a long path multiple reflection IR absorption cell whose path length can be varied. Typical results obtained with this assembly are illustrated in Figure V-2.

b. Plastic Containers

A popular method for studying the atmospheric chemistry and photochemistry of both artificial and natural atmospheric samples uses plastic

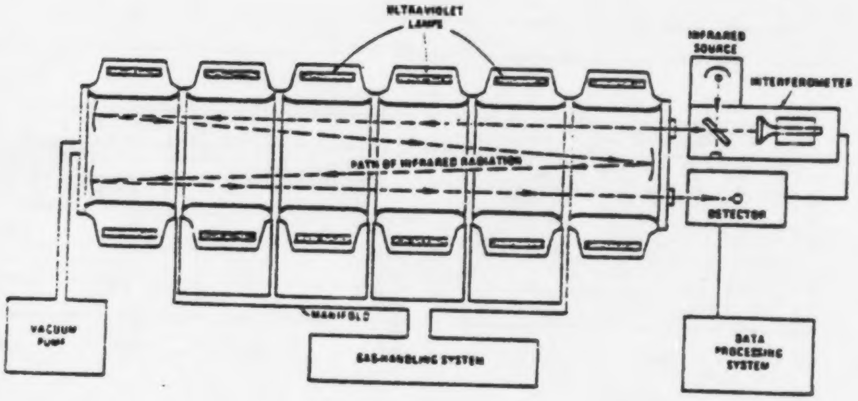
ULTRAVIOLET LAMPS
INFRARED SOURCE
INTERFEROMETER
PATH OF INFRARED RADIATION
DETECTOR
VACUUM PUMP
MANIFOLD
GAS-HANDLING SYSTEM
DATA PROCESSING SYSTEM

Figure V-1. Schematic of long-path infrared photochemical reaction chamber system. (Gay *et al.*, 1976)

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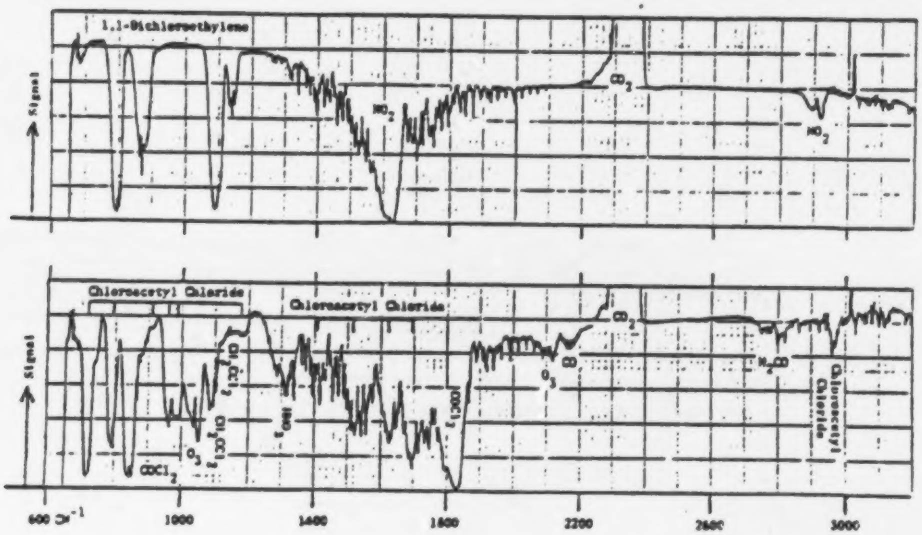

Figure V-2. Spectra of 1,1-dichloroethylene and NO_2. Upper spectrum of 1,1-dichloroethylene (5 ppm) and NO_2 (1 ppm). Lower spectrum after 100 minutes of irradiation [$k_{d(NO_2)} = 0.6\ min^{-1}$, p = 760 torr, 500-m path]. (Gay *et al.*, 1976)

containers. The common bag material is either FEP (Fluorinated Ethylene-Propylene) Teflon, Tedlar or Mylar plastic.

Saltzman and coworkers (1966) used this technique to evaluate the stability of sulfur hexafluoride, bromotrifluoromethane, and octafluorocyclobutane for use as meteorological tracers. The gas being evaluated (SF_6 - 3 to 8 ppb; $BrCF_3$ - 35 to 100 ppb; C_4F_8 - 100 to 500 ppb) was put into the bag with air from a cylinder and small amounts of some of the common reactive pollutants of the atmosphere (H_2O, O_3 - 5.9 ppm, SO_2 - 5 ppm, automobile exhaust 2.8% and H_2S - 5 ppm) were added. Sufficient water to provide a saturated mixture was injected into the bag before addition of the gas mixture. Irradiation studies were carried out with fluorescent black lights (GE F42-T6BL) with 100-liter FEP bags (good transparency in the ultraviolet region). For studies where ozone was incorporated, Mylar bags were used. A control run without the pollutants showed that the gases were slowly lost and this was attributed to diffusion through the 0.002 thick plastic film. No decomposition within experimental error was reported for any of the tracer gases. The possibility of washout by rainfall was checked by placing a bag saturated with water vapor into a 3°C refrigerator. The cold air sample was then drawn into another bag and analyzed. Analysis in all cases was provided by a gas chromatograph (no preconcentration) fitted with an electron capture detector.

Altshuller et al. (1967) used FEP plastic containers to study the photooxidation of hydrocarbons in the presence of aliphatic aldehydes. The reaction mixtures of olefin and aldehyde diluted with air (150 liters) were irradiated with sunlight-fluorescent lamps (maximum intensity at 310 nm) and with natural sunlight. Rates of decomposition of the olefin were determined by gas chromatography using a flame ionization detector.

Kopczynski and coworkers (Altshuller *et al.*, 1970b; Kopczynski *et al.*, 1972) have used plastic containers to measure photochemical reactions of actual atmospheric samples. In the more recent study, they used atmospheric samples collected in a 300 liter Tedlar bag in the early morning (particulates not removed), and irradiation with sunlight was begun as soon as the sample was collected. Duplicate samples were run and a comparison was made between full sunlight and 58% attenuated sunlight. An important step in the procedure was the conditioning of the bag by irradiating an air sample the day before an experimental run. Analysis of the hydrocarbons was conducted by low temperature gas chromatography using a preconcentrated sample and flame ionization detector (GC-FI).

Gay and Bufalini (1971) also used plastic containers in their study of nitrogen balance of irradiated hydrocarbons in the presence of oxides of nitrogen. They used a 100 liter FEP Teflon bag irradiated with GE F42-T6 black lamps with an energy maximum at 366 nm.

A major problem associated with the use of chambers constructed with polyvinyl fluoride (PVF, Tedlar) is that of interference. Van Ham (1978) has shown that PVF releases organic compounds which interfere with measurement of total hydrocarbon concentration, NO_x response, hydrocarbon/NO_x ratio, and the formation of ozone and PAH. The extent of interference was difficult to quantify but it appeared to decrease with increasing surface-to-volume ratio.

c. Glass Flask Reactors

A number of researchers have used glass flask reactors to study atmospheric chemical reactions. The size of the reaction vessel has varied from 100 ml spherical vessels to 72 liter borosilicate flasks.

Wei and Cvetanovic (1963), in a study of the vapor phase reaction of ozone with olefins, used two matched 100 ml spherical reaction vessels made of pyrex glass. These were incorporated into a conventional high vacuum line. The reaction vessels were connected to a gas chromatograph in order to facilitate analysis. A somewhat similar apparatus was used by Wiebe et al. (1973) to study the photolysis of methyl nitrite in nitric oxide, nitrogen dioxide, and oxygen. They used a 500 ml spherical pyrex flask connected in a vacuum line. Irradiation was supplied by a mercury arc lamp (pyrex filters out light below approximately 300 nm). The reaction mixture was analyzed by a mass spectrometer which was connected by a capillary tube to the reaction vessel. Relatively high concentrations of reactants were used (O_3 - 2 mole%; olefins - 20 mole%). The same apparatus was used by Shortridge and Heicklen (1973) to study the chain mechanisms important to the photochemical conversion of NO to NO_2. These authors used a quartz vessel and a variety of light sources including a low pressure mercury lamp (mostly 253.7 nm). The reactions were studied at low pressure (25 torr) and high reactant concentration.

Gay and Bufalini (1971) used two different sized borosilicate flasks as well as a 100 liter plastic bag to study the nitrogen balance of irradiated hydrocarbon and oxides of nitrogen mixtures. A 22 liter borosilicate flask, having a silvered outer surface, was used to determine if molecular nitrogen was a product of photolysis. A mercury lamp (Hanovia No. 679A36) fitted in a double-walled, water cooled, immersion well was used as a light source. Initial concentrations of 60 ppm ethylene and 15 ppm NO_2 in helium or oxygen were used. A similar apparatus, although equipped with a quartz immersion well (Corex filter-cut off at 260 nm), was used by Bentley et al. (1972) to study the photolysis of dimethyl sulfide in air (68 mg/l). These conditions were used to determine the applicability of photochemical methods of controlling odor problems in the paper industry.

The larger borosilicate flask used by Gay and Bufalini (1971) had a capacity of 72 liters. The vessel was placed inside an irradiation chamber described by Altshuller and Cohen (1963). Irradiation was provided by GE F42-T6 black lamps with an energy maximum at 366 nm. The light intensity of the system measured in terms of nitrogen dioxide dissociation was 0.4 min^{-1}. Before each experimental run, the flask was washed with aqueous cleaning solution, 300 ml of acetone, and large amounts of distilled water. The flask was allowed to dry and then equipped with a large magnetic Teflon-finned stirrer and a top with several glass and Teflon lines which served as inlets and exhausts, some of which were connected to gas chromatographs. The flask was then evacuated and refilled with air and the pollutants to be studied. The relative humidity of the tank air was 5%.

Bufalini _et al_. (1972) compared the 72 liter borosilicate flask to a 335 ft^3 smog chamber (Rose and Brandt, 1960) in a study of reasons for poor interlaboratory comparisons of various smog chambers. The 72 liter flask was irradiated in the chamber described above with different light sources (GE F-40-BLB black lights, NO_2 K_d = 0.50 min^{-1} and Westinghouse FS-40 sunlights, NO_2 K_d = 0.14 min^{-1}). The rate of tetramethylethylene photolysis and ozone formation from biacetyl was studied in "clean" and "dirty" flasks. The latter were flasks that were not cleaned with dilute sodium hydroxide solution before each run.

Laity and Maynard (1972) studied the reactivities of gasoline vapors with three types of experimental apparatus: (1) a 400 liter stainless steel chamber, (2) a 235 liter glass vessel, and (3) a 23 liter glass flask. The large glass vessel was a capped, borosilicate-glass, spherical vessel which was placed in a metal box for irradiation. The source of irradiation in all cases was blacklight fluorescent lights. The light intensity is 0.4 min^{-1} (K_d NO_2, 2 ppm in N). Before each run the reaction vessels were cleaned by ozone treatment and evacuation (10^{-2} to 10^{-3} torr, 36 hours, ~60°C).

Stephens and coworkers (Stephens, 1973; Stephens and Burleson, 1967) have used 20 and 50 liter borosilicate carboys to irradiate ambient air samples to study hydrocarbon reactions in air. Irradiation was carried out with both natural sunlight and artificial light (blacklight fluorescent lamps NO_2 K_d 1/2 life = 1.3 min) using a static technique with the 20 liter carboy. When studying aromatics, where the loss of reactant by absorption on the wall was possible, Stephens (1973) used a stirred flow dilution technique. With this technique the air sample in the 50 liter carboy was continuously diluted with dry, hydrocarbon-free, make-up air during the irradiation. No difficulty with adsorption of aromatics was noted. Analysis was provided by gas chromatography combined with a trapping technique in order to make 0.1 ppb detection possible.

A 72 liter borosilicate glass flask of special design has been used by Crosby and Moilanen (1974) to study the vapor phase photolysis of aldrin and dieldrin. The apparatus is depicted in Figure V-3. The flask was painted dull black on the outer surface. The light from the lamp (E in Figure V-3) was passed through the inside of a 59 mm id (27 cm^2 in area) borosilicate glass tubing painted dull black on both the inner and outer surface. The light exited through an unpainted circular area (C) 15 cm in diameter. The removable light trap (D) could be replaced with a 1 liter spherical flask silvered on the interior. A 3 mm-thick borosilicate glass disc (B) prevented escape of vapor and filters out ultraviolet (UV) wavelengths below 300 nm. Inlet and outlet tubes were used only as vents. The lamp was either a 100W low-

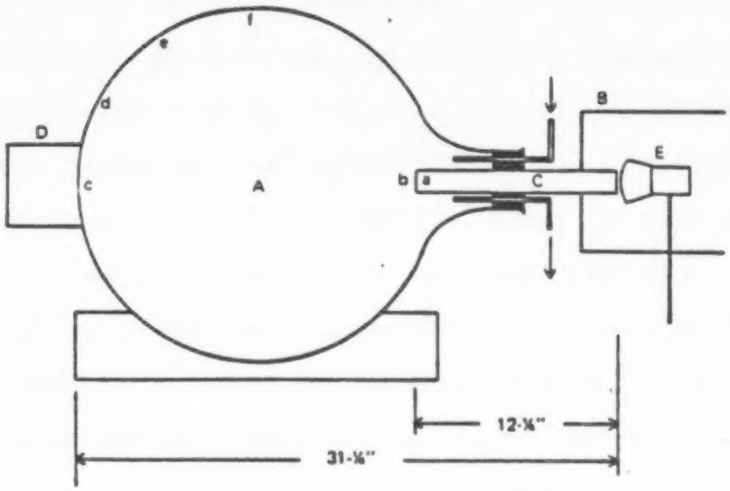
f
e
d
D
c
A
b
a
C
B
E
12-¼"
31-¼"

Figure V-3. Schematic diagram of vapor-phase photoreactor (Crosby and Moilanen, 1974).

pressure mercury lamp (254 nm filtered out, 365, 410, and 440 line) or a 275W RS-sunlamp. In the procedure, the compound to be studied was formed into a thin solid film (hexane evaporation) on a watch glass which was then placed into the bottom of the reaction chamber and warmed to 35°C. After allowing the chemical vapor to evaporate into the chamber (several hours), irradiation was begun. After irradiation for an arbitrary period (45 - 168 hours), the reactor was dismantled and washed with hexane. By comparing results with and without the mirror (varying the light impinging on the inner surface of the reaction chamber), the effect of wall reactions, if any, could be determined. A completely dark control was also run in order to provide evidence that non-photochemical reactions were not taking place.

d. Smog Chambers

There are three major drawbacks to the previously mentioned experimental apparatus for studying atmospheric reactions: (1) their small size allows the possibility of heterogenous reactions, (2) the low ratio of volume to surface allows significant radical termination at the walls, and (3) in some cases the contaminant concentrations have to be unrealistically high in order to provide analytical precision (Doyle, 1970). In order to reduce these difficulties, many researchers have resorted to large photochemical "smog chambers," so named because of their unique capability in studying the phenomena of photochemical smog. The large size of the chamber also allows for simultaneous evaluation of physiological effects such as eye irritation and plant damage.

Most of the smog chambers reported in the literature can be operated in a dynamic or a static mode. The dynamic procedure requires the continuous introduction of reactants and is thought to more closely simulate the dynamic characteristics of the atmospheric environment. It is particularly suited to studies which require large analytical samples. The static procedure consists of batch introductions of reactants into the chamber and because of its simplicity and precision, it is more predominently used (Dimitriades, 1967).

(i) Rose and Brandt Smog Chamber

One of the earliest chamber facilities reported in the literature and one used by EPA (Bufalini et al., 1972) is the environmental irradiation test facility of Rose and Brandt (1960). The facility consisted of five major components: a dynamometer unit, a dilution air purification unit, exhaust dilution assembly, irradiation chambers, and an exposure facility to evaluate air pollution effects of the irradiated pollutants (see Figure V-4). The dynamometer unit ran an internal combustion engine in a way representative of various driving patterns. The exhaust gas was diluted with a clean air supply (cleaned with particulate and charcoal filters) which was maintained at temperatures between 60 and 100°F and at a relative humidity from 35% to 90% by employing a refrigeration air conditioning system. The hydrocarbon concentration was several ppm. The two irradiation chambers, which could be operated in series or parallel, were constructed of aluminum with Mylar Windows and had a volume of 335 ft^3 each.

Circulation in each chamber was maintained by two tube-axial fans. The chambers were designed to approach ideal dilution performance (concentration of output gas would be representative of the entire volume). The average leak loss from one chamber was 0.004 cubic feet/minute (cfm). Irradiation was applied to each chamber by two banks of

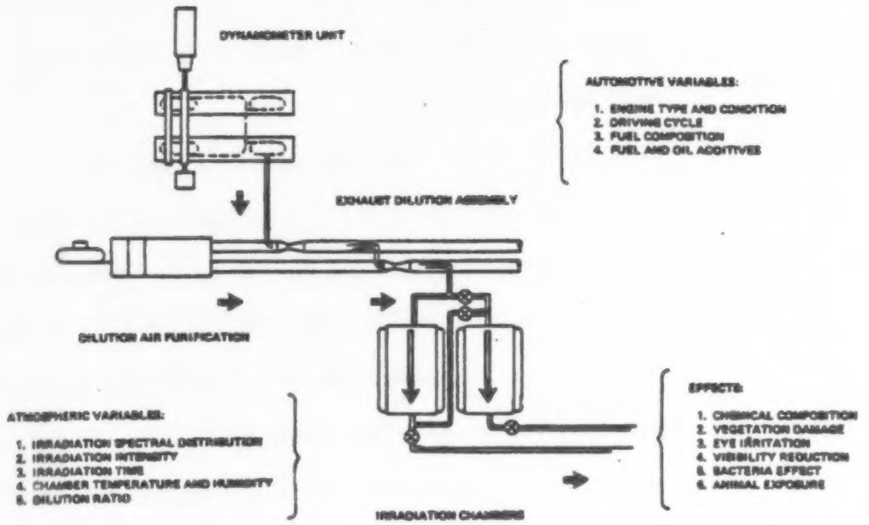
DYNAMOMETER UNIT
AUTOMOTIVE VARIABLES:
1. ENGINE TYPE AND CONDITION
2. DRIVING CYCLE
3. FUEL COMPOSITION
4. FUEL AND OIL ADDITIVES
EXHAUST DILUTION ASSEMBLY
DILUTION AIR PURIFICATION
ATMOSPHERIC VARIABLES:
1. IRRADIATION SPECTRAL DISTRIBUTION
2. IRRADIATION INTENSITY
3. IRRADIATION TIME
4. CHAMBER TEMPERATURE AND HUMIDITY
5. DILUTION RATIO
IRRADIATION CHAMBERS
EFFECTS:
1. CHEMICAL COMPOSITION
2. VEGETATION DAMAGE
3. EYE IRRITATION
4. VISIBILITY REDUCTION
5. BACTERIA EFFECT
6. ANIMAL EXPOSURE

Figure V-4. Environment Irradiation Test Facility (Rose and Brandt, 1960).

70 fluorescent tubes (black, warm white, and blue) in order to simulate solar irradiation from 290 to 450 nm. The exposure time was varied by changing the flow rate (20 to 100 cfm).

When Bufalini and coworkers (1972) used the chamber, they determined the light intensity to be 0.40 min^{-1} in terms of NO_2 dissociation. They studied the oxidation of nitric oxide in the chamber and noted an oxidation rate much faster than theoretical which they attributed to chamber wall contamination.

(ii) Korth, Rose, and Stahman Smog Chamber

Korth and coworkers (1964) in studies conducted at the Taft Sanitary Engineering Center again used a five component facility in a dynamic study of automobile exhaust gases. The irradiation chamber was a 335 ft^3 vessel, presumably the same as used by Rose and Brandt (1960). However, the light intensity was increased (present configuration, Figure V-5) to be more representative of sunlight in the Los Angeles area at a zenith angle of 20 degrees (sunlight curve No. 2, Figure V-5).

Altshuller and coworkers (Altshuller et al., 1969a,b; 1970a) have made extensive use of the Korth et al. (1964) chamber. Although a few experiments have been conducted under dynamic conditions, most of their experiments have used a 6 hour, static-irradiation period. In the 1970 study, a temperature of 31 ± 1° and relative humidity of 50% was maintained. These authors studied the photochemical

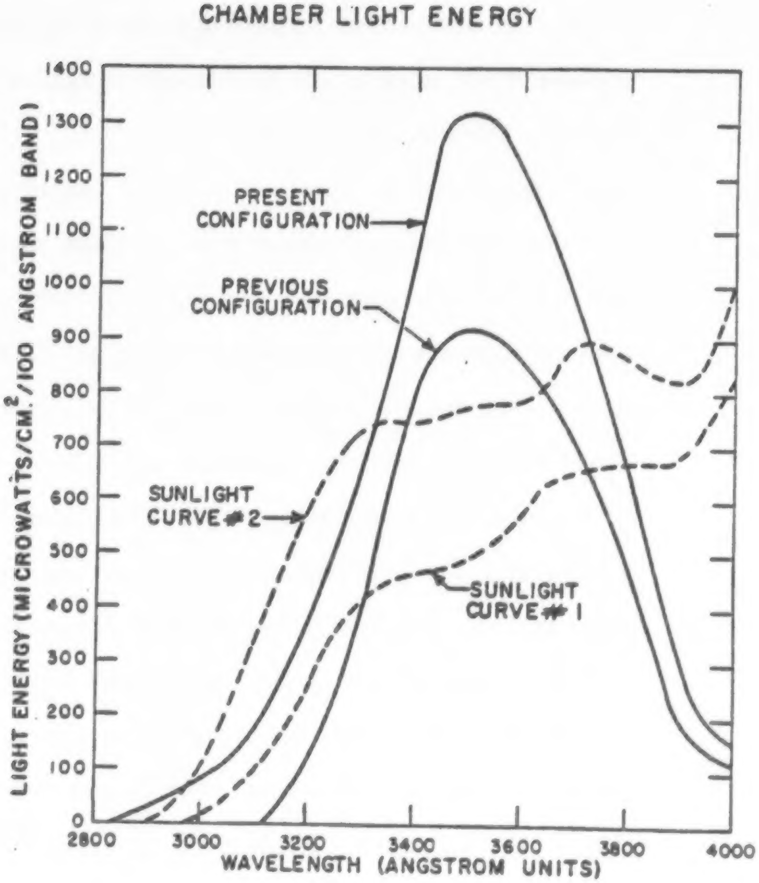

Figure V-5. Chamber light energy for the chamber described by Korth et al. (1964)

reactivities of paraffinic and aromatic hydrocarbons with low concentrations of nitric oxide. Kuntz et al. (1973) used the chamber to study the photoreactivity of benzaldehyde-NO_2 and benzaldehyde-hydrocarbon-NO_x mixtures. They cleaned their make-up air by passage through activated charcoal and particulate filters.

(iii) Bartlesville Petroleum Research Center Smog Chamber

Two irradiation chambers with volume capacities of 64 and 100 ft^3 have been used for air pollution research by the Bartlesville Petroleum Research Center of the U.S. Bureau of Mines (Dimitriades, 1967). The 64 ft^3 chamber was constructed so that the gaseous contents only contacted aluminum and a 5 mm Teflon film. The Teflon film was preferred over the previously used Tedlar (polyvinyl fluoride) because it is less permeable to water vapor and more transparent to ultraviolet light (see Figure V-6). The chamber was equipped with nine sampling ports and was usually run using the static procedure. There were two fans mounted inside for internal circulation. The 100 ft^3 chamber was similar to the 64 ft^3 chamber except that it had pyrex windows. The ozone half-life time at 1 ppm in the small and large chambers with the lights on was 3.6 hours and 6 hours, respectively. The irradiation system consisted of a number of fluorescent lamps designed to produce a light intensity similar to natural sunlight. The K_d NO_2 was 0.38 mn^{-1}. In order to provide analytic samples, 0.7 liters/minute was withdrawn from the chamber and replaced with pure N_2

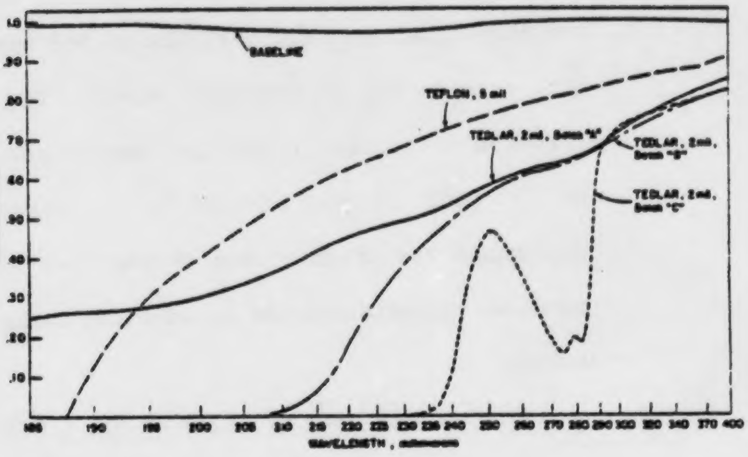

Figure V-6. Spectra of Teflon and Tedlar films in the UV region (Dimitriades, 1967).

(dilution rate 2.3%/hour). Using this apparatus, Dimitriades (1967) studied the effects of background reactivity (chamber reactivity), temperature, and humidity on hydrocarbon-NO_x reactions.

(iv) Stainless Steel Chambers

The General Motors (GM) smog chamber used by Heuss and Glasson (1968) and the smog chamber used by Laity and coworkers (Laity et al. 1973; Laity and Maynard, 1972) were both constructed of stainless steel. The GM chamber consisted of a cylinder 9 feet in diameter and 5 feet high with an internal volume of 298 ft^3. Spaced symmetrically throughout the chamber were 19 vertical borosilicate glass tubes which contained the irradiation lamps; the K_d NO_2 was 0.4 min^{-1}.

The chamber used by Laity et al. (1973) consisted of a polished stainless steel cylinder, 4 feet long and 2 feet in diameter (397 liters-14 ft^3) with blacklight fluorescent lamps mounted inside. Temperature was controlled by circulating liquids around the chamber and between experiments the chamber was cleaned by evacuation and heating (50 to 60°C) overnight. The half-life of ozone (1 ppm) under irradiation varied from 1.3 to 2.5 hours and the intensity of the light was 0.4 min^{-1} (K_d NO_2). The results from the chamber were compared to results obtained from using two glass vessels (235 liters and 23 liters). The following experimental conditions were used: 5 hours irradiation, temperature 32°C, $\sim$1 ppm test chemical, 0.6 ppm nitrogen oxides (0.57 ppm NO, 0.03 ± 0.02 NO_2) and 20% relative humidity.

(v) Stanford Research Institute Smog Chamber

The SRI smog chamber reported by Doyle (1970) is an elaborate facility. The photochemical reaction chamber is mainly constructed of rolled sheets of 7/32 inch Pyrex (transparent down to 300 nm) with some cast aluminum sheet panels. All interior surfaces are coated with Teflon. Sonic jet-type pumps are used to stir the chamber contents (avoids difficulties from impellers). The chamber configuration depicted in Figure V-7 has an internal volume of 7.6 m^3 (269 ft^3). Irradiation is supplied by fluorescent lamps uniformly distributed above the exterior of the chamber. The number and types of lamps were chosen to simulate sunlight intensity from 300 to 600 nm. The longer wavelengths were simulated because the chamber was designed to study compounds that might absorb in that region. The leakage rate from the chamber was 1.5%/hour and the ozone half-life in the irradiated chamber was 6.7 hours. The temperature can be controlled down to 27°C with the lights and the make-up air is humidified to the desired level (usually 35% at 1 atm. and 25°C). Sample ports are built in the chamber for destructive analysis and the optics of a LPIR (40 m path length) are inserted into the chamber through a Teflon-film diaphragm, thus allowing nondestructive analysis.

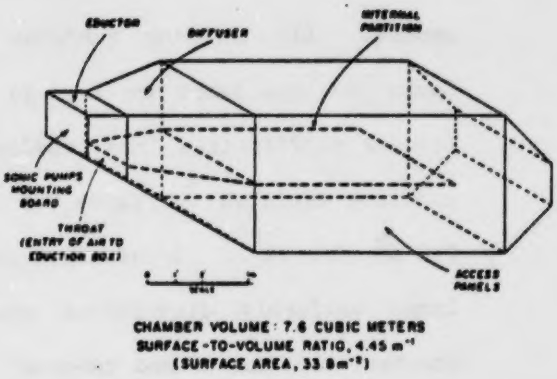
EDUCTOR
DIFFUSER
INTERNAL PARTITION
SONIC PUMPS MOUNTING BOARD
THROAT (ENTRY OF AIR TO EDUCTION BOX)
ACCESS PANELS
SCALE
CHAMBER VOLUME: 7.6 CUBIC METERS
SURFACE-TO-VOLUME RATIO, 4.45 m⁻¹
(SURFACE AREA, 33.8m⁻²)

Figure V-7. SRI smog chamber (Doyle, 1970).

A major effort was made to purify the make-up air. This was accomplished using catalytic combustion over platinum at elevated pressure (5 to 8 atm.) and temperatures (600°C). The pure air supply had the following analysis: <0.5 ppm CO, 0.1 - 0.3 ppm NO_2, and some organic material as analyzed by GC-FI. An 8 hour irradiation of the pure air yielded no detectable oxidant concentrations and little change in the nitrogen oxide concentrations.

(vi) Battelle Memorial Institute Smog Chamber

Wilson _et_ _al_. (1972a,b) have reported the use of the Battelle 610 ft^3 smog chamber made of polished aluminum with Teflon FEP window for the study of a variety of pure organic compounds (α-pinene, cyclohexane, 1-heptene, and toluene). The initial conditions were the following: temperature 88-90°F, relative humidity 39-43%, hydrocarbon - 10 ppm, NO - 0-1 ppm, and NO_2 - 1/2 ppm. Analysis of reaction products was provided by gas chromatography interfaced with mass spectrometry (GC-MS).

Both the SRI smog chamber and the Battelle smog chamber have been used in a comprehensive study of air pollution problems resulting from organic solvent use (Levy, 1973).

(vii) Evacuable and Bakable Smog Chambers

Evacuable and bakable smog chambers were designed to minimize chamber contamination which may lead to irreproducibility (Dimitriades, 1967). The first large evacuable smog chamber was constructed at the University of California at Riverside (Doyle _et_ _al_., 1975) and a comprehensive smog chamber based on the California design has recently been described by Akimoto et al. (1979). A schematic diagram of their design is reproduced in Figure V-8. The chamber is constructed of stainless steel and has a capacity of 6065 liters. The inner walls of the reactor are coated with tetrafluoroethylene-perfluoro alkyl vinyl ether polymer and the coating is applied thinly to reduce gas retention. The chamber walls can be baked at a maximum of 200°C, and temperature control of the chamber is precise to ± 1° within a range of 0-40°C. The irradiation source is a high pressure xenon arc lamp, and the light is filtered through a Pyrex 7740 filter in order to match the spectral distribution of the light to sunlight in the near UV region. Analysis of reagents and products is conducted with a long-path (LP)-FTIR spectrometer, and gas analyzers and chemiluminescent NO_x and ozone analyzers are also included in the assembly.

3. Analytical Procedures

Analytical procedures have considerable influence upon the selection of the reaction conditions used and the results obtained in studies of chemical reactions in the environment. If the sensitivity of the method is low, it may require unrealistically high concentrations or large samples for analysis. Measurement of the reactivity of hydrocarbons and solvents determined by NO loss, NO_2 formation, oxidant formation, hydrocarbon loss, eye irritation, or plant damage can give drastically different results. This section briefly discusses and evaluates the analytical techniques that are used most commonly in atmospheric studies.

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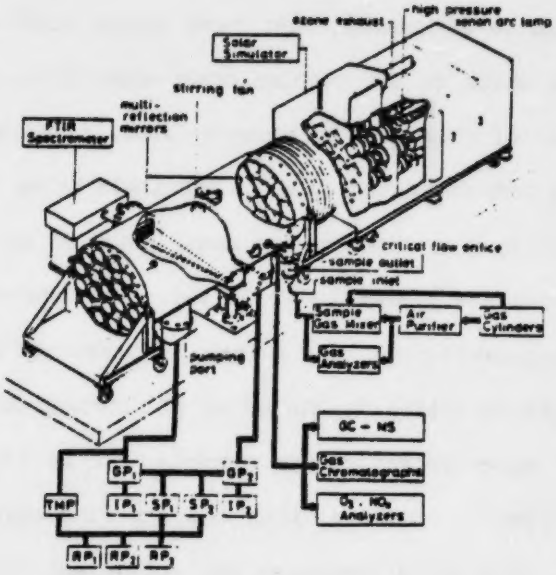

ozone exhaust
high pressure xenon arc lamp
Solar Simulator
stirring fan
multi-reflection mirrors
FTIR Spectrometer
critical flow orifice
sample outlet
sample inlet
Sample Gas Mixer
Air Purifier
Gas Cylinders
Gas Analyzers
pumping port
GC – MS
Gas Chromatographs
O3 . NOx Analyzers
GP1
GP2
TMP
IP1
SP1
SP2
IP2
RP1
RP2
RP3

Figure V-8. Schematic view of the evacuable and bakable photochemical smog chamber system at NIES (Reprinted from Akimoto, et al., 1979. Design and characterization of the evacuable and bakable photochemical smog chamber. Environ. Sci. Technol., 13(4):471-475, with permission of the American Chemical Society.)

a. Long-Path Infrared Spectrometry

When LPIR cells are used as reaction vessels, or when smog chambers are equipped with LPIR windows (Doyle, 1970), the concentrations of the reactants and products are followed by scanning the infrared spectrum. Theoretically any chemical that absorbs between 670 and 5000 cm^{-1} can be monitored. However, in practice a compound must have sharp, high extinction coefficient, diagnostic peaks which do not overlap other absorption bands and this somewhat limits the number of chemicals that can be studied in any one experimental run. These peaks will vary depending upon the chemicals being studied; however, interfering peaks will in most cases be the same. Some of the interfering peaks that have been reported are listed in Table V-1. Fortunately, many compounds have more than one diagnostic peak. For example, Glasson and Tuesday (1971) have used the 1550 cm^{-1} peak to follow the formation of nitrogen dioxide. The interference caused by water vapor absorption was corrected for by monitoring the water peaks at 1560 or 1470 cm^{-1}. However, with the six fluorocarbons that Japar et al. (1974) studied, only three compounds had sufficient diagnostic peaks to allow long duration irradiation. Interferences from relatively inert contaminants (e.g., carbon dioxide) can be removed by using a double-beam (reference and sample cell) infrared spectrometer.

The sensitivity of the method is dependent upon the effective pathlength of the LPIR cell, the intensity of the incident infrared light, and the extinction coefficient of the diagnostic peak. The effective pathlength of the cell is limited by the reflection efficiency of the mirrors. Stephens (1958) has demonstrated that the optimum pathlength is reached when the energy reduced by reflection losses is $\frac{1}{e}$ the initial value. Glasson and Tuesday (1970a,b,c) used a 120 meter pathlength to measure low concentrations of nitrogen dioxide (0.02 ppm) and hydrocarbons (>1.0 ppm). Japar et al. (1974)

Table V-1. Interfering Infrared Absorption Bands from Background Contaminants or Common Products

	Microns
Carbon dioxide*	2.9, 4.3, >15
Water*	2.9, 5.8-7.0, 8-9 some interference, >12
Nitrous oxide*	4.5 (can only be seen if CO_2 is removed)
Nitrogen dioxide***	6.15
Ozone**	9.5
Peroxyacetyl nitrate**	8.6
Formaldehyde**	3.6
Acetone**	8.2
Photooxidation Product Bands**** (isobutylene)	7 - 9
Acetaldehyde****	3.7, 8.9
Methyl nitrate*****	6.25, 7.75, 9.8, 11.72

* Stephens, 1958
** Glasson and Tuesday, 1970a
*** Glasson and Tuesday, 1971
**** Japar _et_ _al_., 1974
***** Tuesday, 1961

reported an experimental error of ±1% (2% concentration error at 50% transmittance) for fluorocarbons at 5 to 8 ppm with a LPIR cell path-length of 40 meters. Modern techniques rely upon Fourier Transform (FT) spectroscopy which offers a considerable enhancement in sensitivity over older continuous wave methods. A good illustration of the application of FT-IR is provided on page V-6.

b. Gas Chromatographic Analysis

Since many of the compounds being studied are volatile organic chemicals (e.g., hydrocarbons), gas chromatography has been extensively used. The simplest application of this technique is the direct injection of the dilute air sample. Bellar et al. (1962) described a continuous sampling system used with a dynamic smog reaction chamber. The system injected 1-cc volumes of air into a gas chromatograph with a flame ionization detector (GC-FI) and had a detection limit of 0.001 ppm for low molecular weight hydrocarbons in complex atmospheric mixtures. Direct analysis of aromatic hydrocarbons in photochemically irradiated mixtures using a 1,2,3-tris(2-cyanoethoxy) propane stationary phase with GC-FI was reported by Clemons et al. (1963). The limits of detection were of the order of 0.01 to 0.1 ppm.

Allen has used a system where 0.1 ml of the cell contents were extracted with a leak free rotary valve and injected immediately into either a GC with a column temperature of -78° to permit separation and detection of CH_4, CO_2, N_2O, NO_2, and NO using helium ionization detection, or into a GC fitted with a capillary column for analysis of organic compounds using flame ionization.

Several researchers have found that direct injection gas chromatographic analysis does not provide the sensitivity necessary (e.g., Altshuller et al., 1970b and Kopczynski et al., 1972) and have resorted to preconcentration of the sample before analysis. Preconcentration or trapping techniques have both advantages and disadvantages. The advantage is that the sensitivity moves into the ppb range. The disadvantages include the need for larger air samples (need larger reaction vessels) and the fact that the procedure is considerably more time consuming. The technique usually consists of trapping the compound of interest on some packing maintained in a "U" shaped cold trap. For analysis the trap is connected to a gas chromatograph and the trap is warmed to release the compound. Bellar et al. (1963) and Stephens and Burleson (1967) reported a sensitivity as low as 0.1 ppb for 100-cm^3 samples using the trapping technique with GC-FI. Jaffe and Smith (1974), using a sampling loop, reported a lower limit of detection of 0.03 ppm for acetaldehyde, total hydrocarbons, and propylene. A relative standard deviation of 10% is likely with this technique (APHA, 1972).

Atmospheric samples have also been analyzed by gas chromatography with electron capture detection (GC-EC) when the compound of interest is more sensitive or more specific to electron capture. Peroxyacetyl nitrate (PAN) is commonly analyzed with this type of detector (Winer et al., 1979). Kopczynski and coworkers (1972) report a sensitivity of 0.1 ppm using a preconcentration procedure with GC-EC. The detector is normally calibrated using a sample from a long-path infrared spectrometer cell which has been analyzed using reported absorptivities. The electron capture detector is also used in the study of halogenated compounds for which the detector is extremely sensitive and specific (e.g., 0.3 ppb for $BrCF_3$; Saltzman et al., 1966).

c. Mass Spectrometry

Because mass spectrometers are able to qualitatively determine reactions species, they have been used to analyze atmospheric reaction mixtures. Wiebe et al. (1973) used a mass spectrometer directly connected to the reaction vessel by a capillary tube to study the importance of NO and NO_2 in scavenging CH_3O radicals. However, this direct interfacing of mass spectrometers has been infrequently used. More common is the separation of the compound of interest followed by mass spectral analysis.

Wilson et al. (1972b) used gas chromatography combined with mass spectrometry (GC-MS) in order to identify specific components of aerosols formed from pure compounds in the Battelle smog chamber. The isolated acid fraction of the aerosol was esterified with diazomethane and then injected into the GC-MS. Analysis of the mass spectrum provided convincing evidence of compound identification. Although the quantities used for analysis are not reported, the amount of material obtained is dependent upon the amount of particulate matter that is filtered. In a dynamically operated smog chamber, this should not be a limiting factor. Several other applications of GC-MS in compound identification have been reported by Nojima et al. (1975), Nojima and Kanno (1977), Kanno and Nojima (1979), and Hendry et al. (1979).

d. Other Methods

Several other methods which are compound specific have been used. The work of Kanno et al. (1977) who irradiated Freon 12 in the presence of NO is a case in point. These authors washed the reaction chamber with water after photolysis, and analyzed the water for the following components: HF by the ion-selective electrode method, HCl by the mercuric thiocyanate method, free chlorine by the o-tolidine method, and formaldehyde by the AHMT method. For the analysis of NO_2, the chamber was washed with 1% triethanolamine in water, and the resulting solution was analyzed by the naphthylethylenediamine method.

HPLC has also been used, albeit infrequently, for the analysis of non-volatile compounds. Kanno and Nojima (1979) photolyzed various chloronitrobenzenes in the presence of air or nitrogen and washed the contents of the reaction vessel with methanol. The unreacted chloronitrobenzenes in the mixture were analyzed by HPLC.

e. Bioassay

In studying atmospheric reactions, physiological analysis is often substituted for chemical analysis because of the poor correlation between chemical analysis and biological effects (e.g., "eye irritation does not give a high linear correlation with any chemical measurement," Laity _et al_., 1973). The two most commonly used physiological effects are plant damage and eye irritation.

For plant damage evaluation, pinto beans, tobacco and petunias have frequently been used to assess the phytotoxicity of gases from irradiation chambers. In the plant exposure chamber used by Korth _et al_. (1964), the plants were uniformly exposed to the irradiated gas for 4 hours and the leaf damage was estimated on the third day (scale of 0-4). Several different responses noted included time of collapse, age of tissue affected, patterns of final injury, and microscopic changes in the progress of tissue collapse. Parameters such as soil type, nutrient content, and light intensity need to be controlled with this technique (Heck _et al_., 1969).

An eye irritation evaluation facility consists of an opening into the reaction chamber up to which panelists can place their eye to determine the irritability of the gas. The facility used by Korth _et al_. (1964) consisted of glass manifolds where five of the ten panelists were exposed simultaneously. Activated charcoal respirators were used to separate odor response from eye irritation response. The degree of eye irritation was rated on a scale of 0-3. The eye irritation ports designed for the SRI smog chamber fit tightly enough that a respirator was not considered necessary (Doyle, 1970). Doyle (1970) has noted that the necessity for simultaneous and uniform exposure in assessing eye irritation requires large chamber facilities.

4. Evaluation of the Techniques

a. General

The techniques that have previously been reviewed have, for the most part, been used to study processes important to the formation of photochemical smog. However, this review is concerned with techniques that can be used to study the persistence and degradation of chemical substances in the atmosphere, and therefore, the evaluation will be directed at this limited question. Evaluation of the techniques for predicting photochemical smog phenomena will be left for others to consider (e.g., Jaffe and Smith, 1974).

"The natural atmospheric environment is a complex, highly reactive system and its simulation requires the duplication of the photochemical and secondary dark reactions involving organic and inorganic substances" (Rose and Brandt, 1960). A number of researchers have suggested that in order to reproduce such a complex system in the laboratory some of the following parameters must be controlled: temperature, humidity, purity of the air supply, concentration of the pollutants, chamber design and operation, spectral distribution and intensity of the light, rate of exchange between chamber and ambient atmosphere, and reaction time (Rose and Brandt, 1960; Levy, 1973; Doyle, 1970; Bufalini _et al_., 1972). The effects of these parameters will be discussed in the following section.

In atmospheric chamber studies, measurements of pollutant disappearance, product formation, visibility reduction, particulate formation, eye irritation, and plant damage are usually recorded. For the purpose of this review, the first two measurements will be considered the most relevant.

b. Factors Affecting Degradation

(i) Spectral Distribution and Intensity of Light

In a comprehensive evaluation of the factors affecting reactions in smog chambers, Jaffe and Smith (1974) compared the reaction rates using a "full" spectrum configuration (>290 nm, xenon arc lamp) to the rates using a "cut-off" spectrum (>350 nm, 3/16" Plexiglass filter with xenon arc lamp). The light intensity was kept constant for both configurations (NO_2 K_d = 0.3 min^{-1}). Of the three independent variables studied (chamber material, spectrum, and surface/volume ratios), the spectral change caused the largest variation; the "cut-off" spectrum clearly slowed the reaction rate relative to the "full" spectrum.

In contrast, Stephens (1973) compared the irradiation of an ambient air sample in a borosilicate carboy with natural sunlight and blacklight fluorescent lamps (simulate sunlight fairly well in the 300-400 nm range, see Crosby, 1969 and Table V-12). The sunlight sample showed more reaction, but the relative reactivities of the hydrocarbons were very similar. This difference in rate may be explained by the difference in intensity (sunlight-brightest day, NO_2 half-life = 1 min; artificial light, NO_2 half-life = 1.3 min) of the different light sources. Furthermore, Laity (1971) concluded that "the differences between blacklight lamps and natural sunlight do not dramatically influence photochemical smog formation with the systems investigated."

Kopczynski et al. (1972) determined the effect of light intensity by irradiating an ambient air sample in a plastic (Tedlar) bag with full sunlight and sunlight attenuated by 58%. They found that the ratio of losses for various hydrocarbons comparing full sunlight to 42% of full sunlight was quite different and concluded that on days of greater intensity the less reactive hydrocarbons would participate more in photochemical reactions in the atmosphere.

(ii) Concentration of Reactants

(a) Humidity: The concentration of water vapor in the atmosphere being studied appears to have a large effect on the reaction rates. Dimitriades (1967) demonstrated a considerable fluctuation in the ethylene reactivity (1.65 ppm as C in 0.50 ppm NO), as measured by rate of NO_2, oxidant, or formaldehyde formation, when the relative humidity was varied from 2.0 - 49.6% (77°F). He concluded that "the need for humidity control in chamber operations is imperative" (Dimitriades, 1967). In a study of the effect of water vapor on the photooxidation of α-pinene, Ripperton and Lillian (1971) observed that increasing the humidity decreased the net mean/time oxidant and O_3 production and net maximum condensation nuclei production, but had an insignificant effect on the average mean/time NO_2, NO, and α-pinene concentrations. Wilson and Levy (1970) have reported that the inter action between SO_2 and photochemical smog is dependent upon the water vapor concentration. In contrast, Bufalini and Altshuller (1969) found no change in the rate of oxidation of nitric oxide (with various hydrocarbons) when the water vapor was increased from 1.1 to 11 mm of Hg. This result conflicts with the water vapor effect noted by Dimitriades (1967). Bufalini and Altshuller (1969) have postulated that the increase in hydrocarbon reactivity caused by the increase in water vapor may be due to a deactivation of active sites on the wall by the water vapor, thus enabling free radicals to have a longer lifetime. They felt that their glass chamber was either conditioned for free-radicals (from prior irradiations) or that the glass system is less reactive than the smog chamber of Dimitriades (1967).

Hydrolysis reactions in the atmosphere effected by water vapor may also be important in some instances. For example, Collier (1972) found that although bis-chloromethyl ether was stable for 18 hours at 10 and 100 ppm in 70% relative humidity air, chloromethyl methyl ether hydrolyzed rapidly.

On the other hand, Butler and Suelson (1979) sutdied the gas phase hydrolysis of three acetyl chlorides and phosgene in the temperature range of 200-350°, and found that hydrolysis occurred at a very slow rate.

(b) NO-Hydrocarbon Concentrations: The atmospheric photooxidation of nitric oxide in the presence of hydrocarbons is the first and one of the most important reactions in the complex series that results in photochemical smog. For this reason, the reaction has received a great deal of study (Altshuller and Cohen, 1963; Altshuller et al., 1969a,b; Glasson and Tuesday, 1970b; Glasson and Tuesday, 1971). Many studies have varied the hydrocarbon and NO concentrations in order to determine the kinetics of the reaction and the differences between studies with individual hydrocarbons in comparison to mixtures. Glasson and Tuesday

(1970a) in a study of the effects of nitric oxide concentration on the photooxidation of propylene, ethylene, trans-2-butene, isobutene, and m-xylene using LPIR, found the following: (1) low concentrations of NO increase the NO photooxidation rate, (2) the rates of hydrocarbon photooxidation reach a maximum at a certain NO concentration, above or below which the HC photooxidation decreases, (3) decreased hydrocarbon concentrations decreased the rate of hydrocarbon disappearance and product formation at all NO concentrations. The initial hydrocarbon concentration has also been found to affect the NO_2 peak concentration, NO_2 dosages, oxidant dosages, peroxyacetyl nitrate formation, and eye irritation (Altshuller et al., 1970a). Glasson and Tuesday (1971) have demonstrated that (a) the nitric oxide photooxidation rates observed with hydrocarbon mixtures are consistently less than the rates calculated from independent experiments with the single hydrocarbon system and (b) rates calculated from linear extrapolations of data obtained with 1.0 ppm of the individual hydrocarbons agree closely with the observed rates for the 1 ppm total HC mixtures. This result is consistent with increased NO inhibition with increase in the total hydrocarbon concentration.

(c) Other Reactants: Besides water vapor, NO_x, and hydrocarbons, a number of other contaminants have been shown to have an impact on atmospheric reactions. For example, Gitchell et al. (1974) have demonstrated the inhibition of NO photooxidation (with hydrocarbons) by phenol, benzaldehyde, and aniline. In a study of the photochemical reactivity of benzaldehyde with NO-HC mixtures, Kuntz et al. (1973) reported a similar decrease in the rate of oxidant formation, hydrocarbon consumption, and NO conversion. Westberg and coworkers (1971) reported that carbon monoxide accelerates the photochemical reaction of isobutene and NO. Wilson et al. (1972a) demonstrated that the presence of SO_2 reduces the maximum NO_2 concentration. Also, sulfur dioxide was found to reduce the maximum oxidant obtained from 1-butene, 1-heptene, and 2,2,4-trimethylpentane, but it increased the oxidant obtained from toluene. Altshuller et al. (1967) have reported that hydrocarbons can be photooxidized with aliphatic aldehydes even in the absence of NO, although the rates are somewhat slower than those induced by NO.

(iii) Temperature

Bufalini and Altshuller (1963) studied the effect of temperature on the photooxidation reaction of trans-2-butene and 1,3,5-trimethylbenzene with nitric oxide in air. The results indicate about a two-fold decrease in conversion times for nitrogen dioxide over the 20° interval from 20°C to 40°C and a corresponding increase in rates of reactions (hydrocarbons disappear almost twice as fast). The authors suggest that the results imply that the temperature changes may be dependent upon the nature of the hydrocarbon. With the trans-2-butene-nitric oxide system, the temperature change has been equated in magnitude to an increase caused by changing the light intensity from $K_d = 0.20$ to $K_d = 0.37\ \text{min}^{-1}$. Doyle (1970) has concluded that these results suggest that the temperature in a chamber should be controlled to within only a few degrees since the lack of precision of most analytical methods would make more precise temperature control useless.

More recently, Carter _et_ _al_. (1979) have studied the effect of temperature variation on smog chamber irradiation of the hydrocarbon-NO_x-air system. Their results indicate that processes such as ozone formation, hydrocarbon and NO_x consumption, and NO to NO_2 conversion are accelerated at elevated temperatures. On the other hand, the ozone forming potential of hydrocarbons is relatively unaffected by temperature. The ozone concentration-time profile exhibited complex behavior with temperature, since processes that form ozone and those that consume it are both temperature dependent. Thus, at lower temperatures, ozone formation generally ceases after NO_2 is depleted, whereas at higher temperatures, the decomposition of PAN can provide a sufficient amount of NO_2 to allow continued ozone formation.

(iv) Chamber Configuration, Construction Materials, and Cleaning Techniques

The importance of surface or wall reactions as they relate to "adsorption on the chamber surface, chemical reaction or decay of gases, chemical reactions catalyzed by the chamber surface, and the formation of new components from the original precursors" has been recognized for a long time (Rose and Brandt, 1960). These processes are probably quite important when considering atmospheric reactions of low volatile materials in reaction chambers. Unfortunately, in most cases, these processes are little understood. However, in an attempt to minimize these effects researchers have varied the construction materials (made as inert as possible), the surface/volume ratio (S/V), cleaning techniques, and chamber configuration. These parameters will be briefly discussed in the following paragraphs.

The shape of the chambers seems to be more dependent upon convenience than upon any suspected effect due to various configuration. The elongated shape of the SRI smog chamber is dictated by construction costs, type of stirring pump (sonic jet-type), and the ability to accomodate a five-membered eye-irritation panel. Jaffe and Smith (1974) used a hexagonal prism shape; Laity and Maynard (1972), cylinder; and Stephens (1973), carboys. A basic consideration is that the chamber contents should be homogeneous, since inhomogeneous mixing can have an effect on the reaction rate (Donaldson and Hilst, 1972). As a result, most chambers contain some type of mixing apparatus. But even the rate of mixing may have an effect as Wilson et al. (1971) have demonstrated for aerosol formation. It is not certain whether aerosol formation is inhibited or whether the aerosol is coated on the fan blades and chamber surfaces (Wilson et al., 1972a).

Cleaning techniques can make substantial changes in the results obtained. An important source of contamination has been reported by Gay and Bufalini (1971) in their study of the nitrogen balance of an irradiated mixture of hydrocarbons and oxides of nitrogen. They found nitric acid as a principal surface product and believed it was formed by the hydrolysis of a nitrogen pentoxide intermediate with water vapor on the wall surfaces. Bufalini _et_ _al_. (1972) suggested that "dirty" chambers may be responsible for the poor quantitative agreement between various smog chambers. They noted drastic differences (no ozone formed) in the irradiation of biacetyl in a clean borosilicate flask (rinsed with base to remove HNO_3) in comparison to results with a flask that had previously been used for biacetyl-NO_x photolysis and not cleaned. With tetramethylethylene (TME) irradiation, they found no reaction in the clean flask, although TME was degraded in an unclean flask (one biacetyl-NO_x-irradiation, evacuated, and then flushed with tank air). These results may also explain the differences observed between irradiation of ambient air samples in conditioned and non-conditioned plastic bags (Kopczynski _et_ _al_., 1972). Conditioning was achieved by irradiating an air sample in a new bag for a full day prior to an experiment. Jaffe and Smith (1974) compared two cleaning techniques (1) purging (4.6 chamber volumes) at 110°F or (2) vacuum off-gassing (2μ pressure or less for 16 hours). The different cleaning techniques had an appreciable effect when used with stainless steel systems.

Contamination problems can be minimized to a large extent in smog chambers that are both evacuable and bakable.

Akimoto et al. (1979) found that baking at 200° under vacuum cleaned the chamber surface, but activated it for the wall decay of O_3 and NO_2. The wall surface could be subsequently deactivated by exposing the chamber to air containing a few percent of O_3 over a two-night period. In all cases, the effect of humidity promoted the surface-catalyzed decay of O_3 and NO_2.

The ratio of a chamber's surface to volume (S/V) can vary considerably (0.78 to 4.91, Jaffe and Smith, 1974). The parameter is directly related to the probability of a molecule in the chamber coming in contact and possibly reacting with the chamber walls. In a study that varied the S/V ratios, Jaffe and Smith (1974) reported that most of the parameters that were measured (e.g., NO_2 formation rate, NO_2 maximum, ozone maximum, etc.) were affected by the S/V ratio for the four materials studied (Teflon, Pyrex, aluminum, stainless steel).

The chamber construction material also appears to be an important parameter affecting atmospheric chamber results. Altshuller and Cohen (1963) attributed the difference between Mylar film and FEP film photooxidation to the difference in light transmittance. However, the inertness of the material may also be important. Laity and Maynard (1972) compared the reactivities of gasoline vapors in a glass and stainless steel chamber and found that the HC-NO_x mixtures always reacted faster in the steel chamber. Jaffe and Smith (1974) compared the effect of the use of Teflon, Pyrex, aluminum, and stainless steel as construction materials. For such reactivity manifestations as NO_2 formation rate, time to NO_2 maximum, ozone maximum, 50% propylene destruction, and NO_2 dose, the following order of reactivity is maintained: Teflon, Pyrex, aluminum, stainless steel. Pyrex and aluminum are similar in behavior for most parameters. Analysis of the data suggests that stainless steel behaves differently than the other materials (Jaffe and Smith, 1974).

c. Internal Consistency of Results

Interlaboratory comparisons of various chambers have shown poor quantitative agreement in results of irradiation of simple hydrocarbon-nitric oxide systems (Bufalini et al., 1972; Jaffe and Smith, 1974). In some cases even results in the same chamber have varied over the years, due to different operation, new light source, etc. (Laity et al., 1973). These differences could not be attributed to analytical variations (Jaffe and Smith, 1974). However, although the quantitative agreement has been rather poor, the qualitative results (ranking or reactivities relative to a standard) can be correlated fairly well as long as the experimental conditions are somewhat similar. Laity et al. (1973) have reported a relative standard deviation of ±10% for these relative rates, even though their chamber performance has varied so much that ten toluene standards were required in the past four years.

Hydrocarbon-NO systems have been studied the most, but only a few studies have determined the relative reactivity measured as hydrocarbon consumption. These have been summarized by Altshuller and Bufalini (1971) and are presented in Table V-2. The determination of hydrocarbon reactivity in terms of nitric oxide oxidation is much more common because of the simple analytical

Table V-2. Ranking of Reactivities of Hydrocarbon Consumption When Photolyzed in Presence of NO Under Static Conditions (Altshuller and Bufalini, 1971)

	Ranking of Hydrocarbons		
Hydrocarbon	Schuck and Doyle (1959)	Stephens and Scott (1962)	Tuesday (1963)
Tetramethylethylene	10	10	10
trans-2-Butene	6		8
cis-3-Hexene		6	
Isobutene	1.5	2	2
1,3-Butadiene	1		
Propylene	1	2	1
m-Xylene		1	
p-Xylene	0.5	0.5	
Ethylene	0.1	0.3	
Hexanes, octanes	<0.1		
Pentanes	<0.1		

technique involved. A comparison of two such studies in a review by Altshuller and Bufalini (1971) showed that the ranking was generally in good agreement and followed the same general order as the relative rates determined by hydrocarbon consumption. A comprehensive study by Glasson and Tuesday (1970b) on hydrocarbon reactivity (measured by NO photooxidation) in the presence of nitric oxide also correlates well with these results.

d. Comparison of Laboratory Results to Behavior in the Natural Environment

Techniques which model atmospheric reactions have little utility if they are not comparable to reactions in the natural environment. This section will briefly discuss the comparability of the results for two chemical groups: (1) hydrocarbons and (2) fluorocarbons. This covers the spectrum of fairly reactive substances to almost totally inert materials.

(i) Hydrocarbons

The reactivity of hydrocarbons has been studied under a variety of laboratory conditions and considerable monitoring data are also available. Evaluating the techniques with a large number of compounds has an advantage in that the relative rates of reaction can be compared without having to resort to the very difficult task of comparing absolute rates. However, if synergistic affects are present in the mixtures, the comparison to pure compound reactivities would be extremely difficult.

Kopczynski et al. (1972) have irradiated with sunlight ambient air samples collected in plastic bags. They compared their data to information obtained from irradiated air samples in glass carboys (Stephens and Burleson, 1967) and relative reactivities determined on pure HC-NO_x mixtures

(Heuss and Glasson, 1968). The relative reactivities, which are presented in Table V-3, are in substantial agreement. Altshuller et al. (1970b) also irradiated with sunlight ambient air samples in plastic bags. They were unable to detect acetylene reaction and the butanes reacted very slowly. However, the relative rates of the other paraffinic and olefinic hydrocarbons were reasonably consistent with other studies.

The studies cited above are still, in a sense, laboratory studies since static conditions were used. In evaluating these results, the more dynamic nature of the open atmosphere must always be kept in mind (Kopczynski et al., 1972). However, direct comparison of ambient air monitoring data to laboratory results is extremely difficult because of varying imputs of different hydrocarbons. Nevertheless, some qualitative comparisons are possible. Stephens (1973) has noted that acetylene, ethylene, and propylene form a unique set of compounds since (1) they are derived almost exclusively from auto exhaust, (2) they are emitted in known ratios to each other, and (3) they differ quite widely in reactivity. If one assumes that the only source of the three compounds is from automobile exhaust, dilution should have no effect on the ratio; only the difference in reactivity should affect the relative concentrations. Table V-4 lists the compound ratios for an air sample taken in the early morning (little photodegradation) and one taken in the late afternoon. The order of reactivity suggested by the

Table V-3. Relative Rates of Percentage Loss of Hydrocarbons Averaged Over Four Hour Irradiation (Kopczynski *et al*., 1972)

	Kopczynski *et al*. (1972)		Stephens and Burleson (1967)	Heuss and Glasson (1968)
Hydrocarbon	Rel. rate	Std. dev.	(4-hr irradn.)	(6-hr irradn.)
Ethane	0.06	0.09		
Propane	0.16	0.09	0.18	
n-Butane	0.27	0.13	0.37	0.11
Isobutane	0.24	0.26	0.33	
n-Pentane	0.37	0.07		
Isopentane	0.43	0.13	0.58	
2,4-Dimethylpentane	0.99	0.27		
Acetylene	0.14	0.07	0.17	
Ethylene	1.00		1.0	1.00
Propylene	2.33	0.42	2.5	1.6
1-Butene	1.62[a]	0.52	2.5	1.9
2-Methyl-2-butene	9.39[b]	1.99		2.1
Toluene	0.56	0.34		0.87
m-Xylene	1.14	0.45		1.2
o-Xylene	0.70	0.28		1.2
sec-Butylbenzene	1.25[c]	0.45		0.47

[a] Contains isobutylene.

[b] Averaged over first hour of irradiation.

[c] Contains 1,2,4-trimethylbenzene.

Table V-4. Comparison of Acetylene, Ethylene, Propylene Ratios of Two Ambient Air Samples (derived from data in Stephens, 1973)

	Hydrocarbons in Morning Air (0735-0800 hr) (ppb)	Ratio $C_2H_2 = 1.00$	Hydrocarbons in Polluted Air (1500 hr) (ppb)	Ratio	% Change in Ratio
Acetylene	78	1.00	27	1.00	--
Ethylene	64	0.82	16	0.59	27%
Propylene	14	0.18	1	0.04	78%

monitoring data (ethylene>propylene) is consistent with reactivities derived under static conditions in the laboratory.

(ii) Fluorocarbons

Fluorocarbons are released to the environment in large quantities mainly due to their use as aerosol propellants. Three studies under laboratory conditions of the atmospheric stability of these compounds are available. Saltzman et al. (1966) examined bromotrifluoromethane and octafluorocyclobutane as potential meteorological tracers by exposing the compounds (100-250 ppb) in a plastic bag to light, ozone, water vapor, SO_2, and diluted automobile exhaust. They found no reactivity. However, these compounds are not commercially important and no ambient monitoring data are available. Japar et al. (1974) examined the photostability of fluorocarbon 11 (CCl_3F), 12 (CCl_2F_2), 22 ($CHClF_2$), 113 ($Cl_2FCCClF_2$), 114 (ClF_2CCClF_2), and 115 (ClF_2CCF_3) in a LPIR instrument in the presence of nitric oxide and an olefin. For irradiations lasting as long as eight hours, no decrease in fluorocarbon concentration was noted. Hester et al. (1973) irradiated CCl_2F_2 and CCl_3F in the absence of hydrocarbons for periods of up to two months and found no degradation.

Lovelock et al. (1973) have detected CCl_3F levels of 49.6 ppb (by volume) above the Atlantic Ocean, and Su and Goldberg (1973) report concentrations of 97 ppb and 700 ppb of CCl_3F and CCl_2F_2, respectively, in a desert area well removed from industrial sources. Based upon the above monitoring data and estimates of fluorocarbon released to the atmosphere, the above authors calculated minimum residence times of 10 (CCl_3F - Lovelock et al., 1973) to 30 years (CCl_2F_2 - Su and Goldberg, 1973). Obviously, the atmospheric stability of fluorocarbons based both on the laboratory studies and monitoring data is in good qualitative agreement.

e. General Discussion of the Advantages and Disadvantages of the Various Methods

The choice of a technique to study the atmospheric reactivity of a chemical substance has both technical and economic trade-offs. This section will discuss only technical considerations.

In general, the techniques that have been reviewed have been designed to study relatively short term (hours) phenomena. For example, although 94% of trichloroethylene reacts in 6 hours of irradiation, trichloroethylene has been characterized as "moderately reactive" (Altshuller and Bufalini, 1971). The applicability of these techniques to studies of long term processes (days, weeks, months), which are important to a determination of the persistence and fate of a chemical substance, is unknown. In addition, with the exception of the study by Crosby and Moilanen (1974), only compounds with high vapor pressures have been studied.

Chamber studies can be conducted under either dynamic or static conditions. The advantages and drawbacks of these two procedures are noted in Table V-5. The size of the chamber will effect (1) the surface/volume ratio and (2) the analytical sample available and thus the concentration of reactants that must be used. The construction material can effect both the wavelength and intensity of the light source and the ability of the chamber walls

Table V-5. Advantages and Disadvantages of Static vs. Dynamic Procedures in Studying Atmospheric Reactions (Dimitriades, 1967; Doyle, 1970)

Dynamic		Static	
Advantage	**Disadvantage**	**Advantage**	**Disadvantage**
Better approximates the atmospheric situation Lower effective surface/volume ratio (lower chance of wall reactions) Larger analytical samples-use lower, more realistic concentrations Large samples for eye irritation and plant damage studies	Instability of flow and composition introduces source of experimental error Need elaborate dilution, purification, and air handling system Limited time of irradiation	Simple and reliable More tractable, better precision Simplifies chemical reaction system	Less like the dynamic atmosphere Higher effective surface/volume ratio Smaller analytical sample-need higher concentrations

to terminate radical reactions. The methods that have been used for studying atmospheric reactions will be reviewed in the following paragraphs.

Long-path infrared (LPIR) systems require a minimum of analytical development time since most compounds have a diagnostic infrared absorption. However, because of the analytical technique, the systems studied have been relatively simple (mostly HC-NO systems) and, unless one resorts to Fourier Transform IR, the concentrations of reactants are somewhat higher than what would normally be found under ambient conditions. The use of stainless steel with fluorescent lights on the inside of the chamber (Glasson and Tuesday, 1970a,b,c) seems somewhat less desirable than using more inert substances, such as Teflon (Japar _et_ _al_., 1974), with the light source on the outside.

The use of plastic containers appears to be a versatile method of irradiating both artificial and natural air samples with sunlight or artificial light sources. FEP Teflon seems to be the best material because of its UV transparency. Analytical development of methods of analysis for the low concentrations that would be desirable could be quite time consuming.

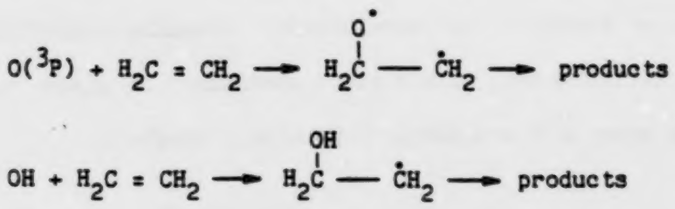
Similar considerations apply to the use of glass flask reactors with the expectation that the glass reactors should be easier to rinse out for either sampling or cleaning.

Smog chambers have the advantage of larger size (lower surface/volume ratio, larger analytical sample-lower concentrations of reactants) and the potential for dynamic operation. In addition, it is easier to maintain control of humidity and temperature. Cleaning operations which were previously more difficult than with plastic containers or glass reactors may now be conducted with ease with evacuable and bakable chambers.

5. Reactivity Scale for Atmospheric Hydrocarbons

Of the several oxidizing species such as O_3, O, OH, and HO_2, which are capable of oxidizing organic hydrocarbons in the atmosphere, the hydroxyl (OH) radical has been found to be the most reactive. Darnall et al. (1976) have correlated the rates of hydrocarbon disappearance in smog chamber studies with hydrocarbon-OH radical rate constants (Figure V-9), and have constructed an index (Tables V-6 and V-7) for hydrocarbons based on their reactivity towards the OH radical. Extrapolation of the index to environmental situations is expected to be fairly accurate for alkanes and aromatics, since the rates of reaction of these hydrocarbons with oxidants such as ozone, $O(^3P)$ atoms, and hydroperoxyl radicals are much lower than those obtained with the hydroxyl radical. By contrast, the extrapolation is less appropriate for alkenes which react with ozone, or for compounds such as aliphatic aldehydes which can undergo photodissociation in the atmosphere. The hydroxyl radical rate constants have also been correlated with ozone formation (Winer et al., 1979), and it is likely that measurement of these rate constants will be a major step towards defining atmospheric persistence.

Measurement of hydroxyl radical rate constants may be made in one of two ways (see following section), and may also be estimated through linear free energy relationships. Gaffney and Levine (1979) have reasoned that since the reaction of $O(^3P)$ and OH with alkenes constitute addition processes as shown below,



$$O(^3P) + H_2C = CH_2 \longrightarrow H_2\overset{\overset{\displaystyle O^\bullet}{|}}{C} - \dot{C}H_2 \longrightarrow \text{products}$$

$$OH + H_2C = CH_2 \longrightarrow H_2\overset{\overset{\displaystyle OH}{|}}{C} - \dot{C}H_2 \longrightarrow \text{products}$$

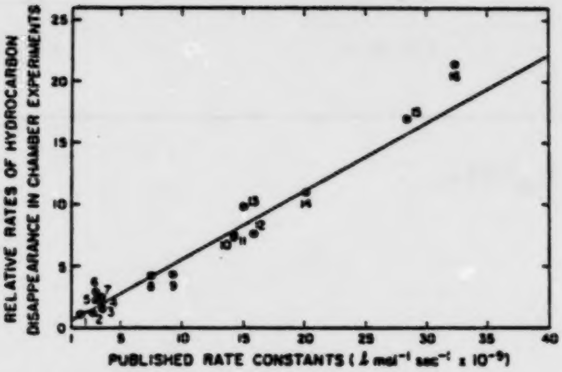


Figure V-9. Comparison of relative rates of hydrocarbon disappearance determined by environmental chamber method (Doyle et al., 1975; Lloyd et al., 1976) with selected published rate constants (cited in Table V-7) for reaction of those hydrocarbons with OH radicals. Line shown represents one to one correspondence and has slope of (1/1.8) $\times 10^{-9}$ mol s $1.^{-1}$. Compounds shown are: 1,n-butane; 2,isopentane; 3, toluene; 4, 2-methylpentane; 5, n-hexane; 6, ethylene; 7,3-methylpentane; 8, p-xylene; 9, o-xylene; 10-11, m-xylene; 12, 1,2,3-trimethylbenzene; 13, propene; 14, 1,2,4-trimethylbenzene; 15, 1,3,5-trimethylbenzene; 16, cis-2-butene. (Darnall et al., 1976)

Table V-6. Reactivity Scale for Hydrocarbons Based on Rate of Disappearance of Hydrocarbon Due to Reaction with Hydroxyl Radicals (Darnall *et al.*, 1976)

Class	Half-life[a]	Reactivity Relative to Methane (=1)
I	>9.9 days	< 10
II	24 h to 9.9 days	10-100
III	2.4-24 h	100-1000
IV	0.24-2.4 h	1000-10,000
V	<0.24 h	> 10,000

[a] $t_{1/2} = 0.693/k_{OH}[OH]$.

Table V-7. Proposed Reactivity Classification of Hydrocarbons and CO Based on Reaction with Hydroxyl Radicals (Darnall *et al.*, 1976)

Compound	k_{OH} + Cpd (1. mol^{-1} s^{-1}) x 10^{-9}	Reactivity Relative to Methane	Proposed Class (see Table V-6)
Methane	0.0048	1	I
CO	0.084	18	II
Acetylene	0.11	23	II
Ethane	0.16	33	II
Benzene	0.85	180	III
Propane	1.3	270	III
n-Butane	1.8	375	III
Isopentane	2.0	420	III
Methyl ethyl ketone	2.1	440	III
2-Methylpentane	3.2	670	III
Toluene	3.6	750	III
n-Propylbenzene	3.7	770	III
Isopropylbenzene	3.7	770	III
Ethene	3.8	790	III
n-Hexane	3.8	790	III
3-Methylpentane	4.3	900	III
Ethylbenzene	4.8	1000	III-IV
p-Xylene	7.45	1530	IV
p-Ethyltoluene	7.8	1625	IV
o-Ethyltoluene	8.2	1710	IV
o-Xylene	8.4	1750	IV
Methyl isobutyl ketone	9.2	1920	IV
m-Ethyltoluene	11.7	2420	IV
m-Xylene	14.1	2920	IV
1,2,3-Trimethylbenzene	14.9	3100	IV
Propene	15.1	3150	IV
1,2,4-Trimethylbenzene	20	4170	IV
1,3,5-Trimethylbenzene	29.7	6190	IV
cis-2-Butene	32.3	6730	IV
beta-Pinene	42	8750	IV
1,3-Butadiene	46.4	9670	IV-V
2-Methyl-2-butene	48	10,000	[illegible]
2,3-Dimethyl-2-butene	67	14,000	V
d-Limonene	90	18,800	V

the rates of reaction should correlate with the ionization potentials of the corresponding alkenes. Gaffney and Levine (1979) showed this to be the case, and also demonstrated that rates for $O(^3P)$ reactions could be correlated with those for the corresponding OH reactions.

For alkanes, $O(^3P)$ and OH attack occurs via abstraction reactions as illustrated below,

$$O(^3P) + RH \longrightarrow OH + R\cdot \longrightarrow \text{products}$$

$$OH + RH \longrightarrow H_2O + R\cdot \longrightarrow \text{products}$$

and these rates were found to correlate with bond dissociation energies, although the correlations were not as good as those obtained for the alkenes. In any event, rates of OH attack on a variety of substrates may be calculated using the correlation, and the atmospheric persistence of these compounds may therefore be estimated.

a. Measurement of the Rates of OH Radical Attack on Organic Compounds

The rates of OH radical reaction with atmospheric contaminants are of fundamental importance for the assessment of environmental persistence, and several of these measurements have recently been reported. In general, the measurements fall into two broad categories: (i) where OH radical concentration is directly monitored in the presence of excess substrate and (ii) where a mixture of compounds, one or more of which are standards, are reacted with OH radicals, and the relative rates are obtained by measuring reactant and product concentrations. These rates are converted to absolute rates by using the known rates of reaction of the standard compounds with the OH radical. Table V-8 exemplifies some of the more recent kinetic studies.

Table V-8. A Summary of Representative Photochemical Kinetic Studies

Compound	Primary Species Monitored	Analytical Technique	Reference
Halocarbons	OH	Resonance absorption	Handwerk and Zellner, 1978
Halocarbons and Hydrogen	OH	Resonance absorption	Atkinson *et al.*, 1975
CO, CH_4	OH	Resonance fluorescence	Davis *et al.*, 1974
C_2H_4, C_2H_2	OH	Resonance fluorescence	Davis *et al.*, 1974
C_2H_2	OH	Absorption photometry	Pastrana *et al.*, 1974
Aldehydes	reactants	LP-FTIR	Niki *et al.*, 1978
Various Hydrocarbons	reactants	GC	Lloyd *et al.*, 1976
Halocarbons	NO_x	Chemiluminescence	Cox *et al.*, 1976
Dimethyl Sulfide	OH	Resonance fluorescence	Kurylo, 1978
OCS, CS_2	OH	Resonance fluorescence	Kurylo, 1978
Aromatic Hydrocarbons	OH	Resonance fluorescence	Hansen *et al.*, 1975
CO, C_2H_4, C_2H_2	OH	Resonance absorption	Smith and Zellner, 1973
Alkyl and Aryl Hydrocarbons	O_3 NO_x CO, PAN, reactants	UV Absorption Chemiluminescence GC	Winer *et al.*, 1979
Halocarbons	OH	Resonance fluorescence	Chang and Kaufman, 1977

It is likely that the relative rate approach will be used to an increasingly greater extent since several compounds may be studied simultaneously by this technique, and accurate absolute rates for a number of standard compounds are available. An example of the method is provided by Lloyd et al. (1976) who examined a variety of hydrocarbons in chamber experiments. n-Butane was included as a standard in all their experiments, and their results, illustrated in Table V-9, suggest that rate measurements precise to within ±20% may be made routinely by this technique.

b. Products from OH Radical Reactions

Owing to its high reactivity, the OH radical may react with organic substrates or intermediates in a variety of ways. Saturated hydrocarbons suffer hydrogen abstraction, whereas unsaturated substrates are generally attacked at the site of unsaturation (Margitan et al., 1974).

$$\cdot OH + CH_4 \longrightarrow \cdot CH_3 + H_2O$$

Sloane and Brudzynski (1979) have explored the extent of site selectivity in the reaction of OH radicals with phenylacetylene and styrene. These authors generated OH radicals from the reaction of H atoms with NO_2 and studied the products of the hydrocarbon-OH radical reaction with a mass spectrometer interfaced to the reaction chamber. For phenylacetylene, OH radical attack occurred on the terminal alkynyl carbon and on the aryl ring. The carbon atom attached to the ring was unreactive, as expected from frontier electron density calculations. In contrast, both side chain carbon atoms in styrene were reactive, as was the ring itself, towards OH radical attack.

Table V-9. Rates of Disappearance and Rate Constants for Selected Alkanes, Alkenes, and Aromatic Hydrocarbons at 1 atm in Air at 305 ± 2 K

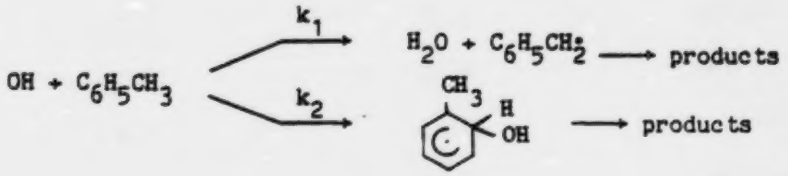
Compound	Relative rate of disappearance	$10^{-9}k$,[a] $M^{-1}\ s^{-1}$
n-Butane	1	1.8[b]
Isopentane	1.10	2.0 ± 0.4
2-Methylpentane	1.77	3.2 ± 0.6
3-Methylpentane	2.40	4.3 ± 0.9
n-Hexane	2.09	3.8 ± 0.8
m-Xylene	7.18	12.9 ± 2.6
n-Propylbenzene	2.07	3.7 ± 0.8
Isopropylbenzene	2.03	3.7 ± 0.8
Ethylbenzene	2.65	4.8 ± 1.0
o-Ethyltoluene	4.57	8.2 ± 1.6
m-Ethyltoluene	6.49	11.7 ± 2.3
p-Ethyltoluene	4.33	7.8 ± 1.6
Ethene	2.88	5.2 ± 1.0
Propene	9.70	17.5 ± 3.5
1,3-Butadiene	25.8	46.4 ± 9.3
cis-2-Butene	21.8	39.2 ± 8.0

[a] The indicated error limits are ± 20% and are the estimated overall error limits.

[b] Placed on an absolute basis using the mean of the literature values.

Hendry et al. (1979) have also attempted to interpret aromatic hydrocarbon-OH radical reaction products from a mechanistic viewpoint. In their experiments, substrate concentrations were maintained in excess of OH radical concentrations to minimize secondary reactions, and products were monitored by GC-MS.

For toluene, the reaction products included benzaldehyde, benzyl alcohol, 3-nitrotoluene, 2, 3, and 4 cresols, and 2-methyl-1,4-benzoquinone. These were characterized by GC/MS and were postulated to arise via two primary processes: abstraction of H• from toluene to give the side chain substituted compounds, and radical addition to the ring to yield ring substituted products.



Since k_1 and k_2 represent parallel reactions, the rate ratio $k_1/(k_1 + k_2)$ should equal [side chain products]/[total products]. This was found to be the case since the product ratio (0.15) corresponded to the ratio (0.14) which was independently measured by Perry et al. (1977). Similar results were obtained for benzene, 1,4-dimethylbenzene, and 1,3,5-trimethylbenzene.

For the reaction of •OH with benzaldehyde, the only gaseous product was phenol. The absence of any ring-substituted products was in accordance with the known electrophilic nature of •OH. The formyl group in benzaldehyde is electron withdrawing, and would slow the rate of ring addition relative to that of side chain abstraction. This difference in reactivity would manifest itself in the reduction or elimination of ring-substituted products as observed.

The fate of halocarbons varies with the degree of halogen substitution. For saturated compounds which contain hydrogen, the hydrogen is abstracted by OH radical to give a halocarbon radical which then reacts further (Chang and Kaufman, 1977; Watson et al., 1977). Fully halogenated substrates are consequently unreactive towards OH radicals, and compounds such as CF_4 and $CF_2ClCFCl_2$ are stable in the atmospheric environment (Cicerone, 1979; Watson et al., 1977).

B. Photochemical and Chemical Alterations in the Aqueous Environment

1. Photochemical Alterations

a. Introduction

Chemically stable non-volatile compounds that are released to the environment eventually reside in the soil and water. Photochemical processes that occur in these media depend upon the degree of attenuation of incident sunlight and are therefore bound by constraints of wavelength and intensity. For example, in soil, only compounds that reside at the very top are susceptible to photolysis. Although water is transparent to sunlight (transmits light > 180 nm), the intensity falls off with increasing depth, and below a few meters photolysis probably proceeds at negligibly small rates (Crosby, 1972; Zepp and Cline, 1977). Nevertheless, photolysis represents an environmentally important degradative pathway for those compounds that are photoreactive but otherwise relatively inert, and a considerable body of data pertaining to photochemical pathways, processes, and mechanisms is available. Table V-10 lists a number of the more recent photolysis studies conducted from an environmental viewpoint along with the chemicals studied, the conditions, and the photoprocesses involved.

Table V-10. Aqueous and Soil Photochemical Studies

Compound	Physical State or Solvent	Light Source	Wavelength	Analytical Procedure	Process	Reference
Sustar	Aq. solution	sunlight, blacklight	>290	GC, ^{14}C-TLC	oxidation, other	Miller and Crosby, 1978
Naphthalene	Aq. solution	450 W medium pressure lamp	313	HPLC	--	Zepp and Schlotzhauer, 1979
Anthracene	Aq. solution	450 W medium pressure lamp	313	HPLC	--	Zepp and Schlotzhauer, 1979
Pyrene	Aq. solution	450 W medium pressure lamp	313	HPLC	--	Zepp and Schlotzhauer, 1979
Naphthacene	Aq. solution	450 W medium pressure lamp	313	HPLC	--	Zepp and Schlotzhauer, 1979
Benz[a]anthracene	Aq. solution	--	--	--	--	Smith et al., 1977
Benz[a]pyrene	Aq. solution	--	--	--	--	Smith et al., 1977
Benzidine	Aq. solution	sunlight, 450 W medium pressure lamp	>290 nm	HPLC	reductive dechlorination	Banerjee et al., 1979
3-Chlorobenzidine	Aq. solution	sunlight, 450 W medium pressure lamp	>290 nm	HPLC	reductive dechlorination	Banerjee et al., 1979
3,3'-Dichlorobenzidine	Aq. solution	sunlight, 450 W medium pressure lamp	>290 nm	HPLC	reductive dechlorination	Banerjee et al., 1979
Hexazinone	Aq. solution	sunlight	>290 nm	^{14}C-TLC	demethylation	Rhodes, 1980
DDA	Aq. solution	sunlight	>290 nm	HPLC	oxidation	Ware et al., 1980
Pentachlorophenol	Aq. solution	sunlight	>290 nm	GC	dechlorination, hydroxylation	Wong and Crosby, 1978
Monuron	Aq. solution	450 W medium pressure lamp	>290 nm	TLC	hydroxylation, oxidation	Tanaka et al., 1977
Cytrolane	Aq. solution	sunlight	>290 nm	GC-MS	hydrolysis	Ku et al., 1979
Diphenyl ether herbicides	Aq. solution or suspension	sunlight	>290 nm	GC	dehalogenation, other	Ruzo et al., 1980
BHT	Aq. solution	sunlight	>290 nm	^{14}C-TLC	oxidation	Mikami et al., 1979
Methoxychlor	Aq. solution	sunlight	>290 nm	GC	dehydrochlorination	Zepp et al., 1976
Fluridone	Aq. solution	sunlamps, blacklight	>290 nm	GC	--	West et al., 1979
Nitrapyrin	Aq. solution	RPR-100	>290 nm	GC	hydrolysis, hydroxylation	Meikle et al., 1978
Iralec	Methanol	RPR-300	290-310 nm	GC	nucleophilic substitution	Sundstrom et al., 1977
N-Nitrosodi-n-propylamine	Aq. solution	sunlight	>290	GC	N-N cleavage	Saunders and Mosier, 1980

Table V-10. Aqueous and Soil Photochemical Studies (Cont.)

Compound	Physical State or Solvent	Light Source	Wavelength	Analytical Procedure	Process	Reference
Butyl benzyl phthalate	Aq. solution	sunlight	>290	GC	--	Gledhill, 1980
Triphenyl tin hydroxide	Aq. solution	sunlight	>290	GC	Sn-C cleavage	Soderquist and Crosby, 1980
Halobenzenes	Aq. acetonitrile	--	>280 nm	--	dechlorination, dimerization	Choudhry, 1979
5-Chlorouracil	Aq. solution	white fluorescent light	>320	UV	--	Southworth and Gehrs, 1976
Mercaptopyridine-N-oxide biocides	Aq. solution	sunlight	>290	UV	--	Neihof et al., 1979
Polychlorodibenzo-p-dioxins	hexane	sunlight	>290	GC	dechlorination	Dobbs and Grant, 1979
Chlorothalonil	Benzene, Hexane, Acetone	sunlight	>290	GC	phenylation	Saton, 1979
2,4-D	Aq. solution	sunlight, 450 W medium pressure lamp	>290	GC	hydroxylation, other	Zapp et al., 1975
3,4-Dichloroaniline	Aq. solution	sunlight	>290	GC	sensitized dimerization	Miller et al., 1980
Bromacil	Aq. solution	sunlight	>290	GC	sensitized	Acher and Dunkelblum, 1979
TCDD, Trifluralin	adsorbed to soil or silica	sunlight	>290	^{14}C-TLC	--	Plimmer, 1978
Organophosphorus insecticides	Aq. solution, adsorbed to soil	Xenon lamp	>290	GC, ^{14}C-TLC	hydrolysis	Burkhard and Guth, 1979
Isopropyl-3-Chlorocarbanilate	Aq. solution	high pressure mercury lamp	>280	HPLC, ^{14}C-TLC	hydroxylation	Guzik, 1978
Isopropyl-3-Chlorocarbaonilate	Aq. solution, 2% acetone	high pressure mercury lamp	>280	HPLC, ^{14}C-TLC	oxidation	Guzik, 1978
Chlorinated p-terphenyls	methanol, cyclohexane	RUL 3000	300	GC	dechlorination, substitution	Chittim et al., 1978
Pentachlorophenol	Aq. solution	sunlight	>290	GC	hydroxylation, other	Wong and Crosby, 1981

b. Techniques Used to Determine Photoalterations

(i) Light Sources

Perhaps the most crucial factor affecting the photoproducts and their rates of formation is the wavelength and intensity of the light source. Consideration must be given to both the light source and any media that the light passes through before irradiating the test chemical.

The use of actual sunlight best represents environmental conditions, and therefore, sunlight is frequently used as a light source. For relatively photostable chemicals, however, where long exposure times are required, many workers have resorted to artificial light sources.

Arc and fluorescent lamps are by far the most popular artificial light sources because they provide a rich source of UV light. The mercury arc lamp in which an electric discharge is generated in gaseous mercury vapor has become the standard UV source for most photochemical research. The arc can be operated at high, medium, and low pressure and this results in a series of sharp spectral lines (low pressure) or a fairly evenly dispersed irradiation intensity over a broad wavelength range (high pressure) as is depicted in Figure V-10 (Calvert and Pitts, 1966).

The higher pressure mercury lamps radiate much of the energy as heat (>80%) (Crosby and Li, 1969), and therefore precautions must be taken to prevent overheating of the chemical being photolyzed and to protect against glass breakage. The low pressure lamps, sometimes referred to as "germicidal lamps," are cooler and far more efficient (over 90% emitted as light) but most of

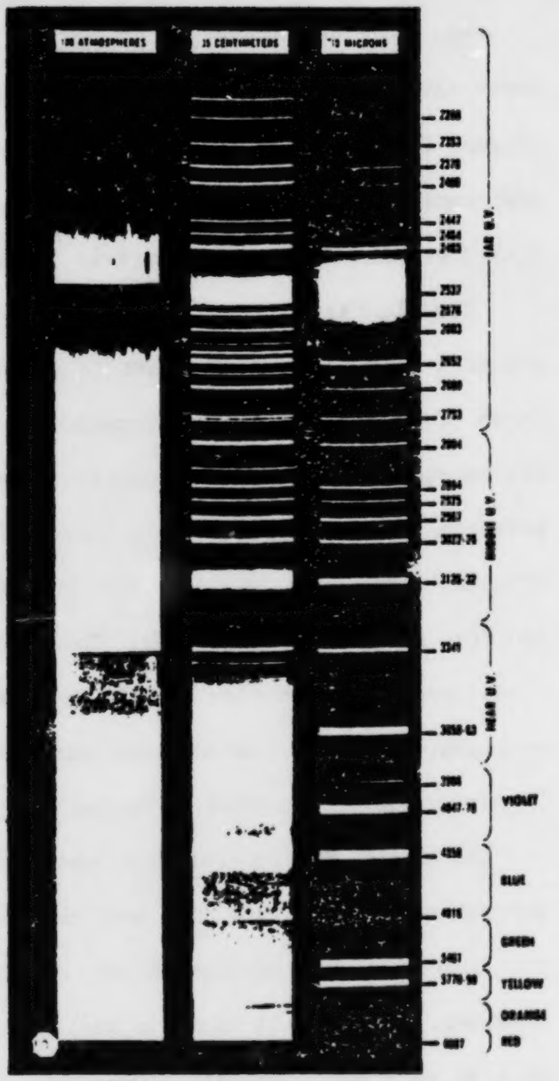


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Figure V-10. Emission spectrum of the low-pressure (10µ), medium pressure (35 cm), and high pressure (100 atm) mercury arcs (Calvert and Pitts, 1966).

their intensity is concentrated at 253.7 nm, which is far below the wavelength energies found in sunlight.

The most environmentally relevant of the arc lamps is the xenon lamp whose spectrum closely approximates that of sunlight (Figure V-11). While these lamps have not been used in previous environmental studies to any appreciable extent, it is likely that they will be used extensively in the future.

The fluorescent lamp is a well-known variation of the low-pressure mercury arc. The lamp is internally coated with a thin layer of mineral or organic phosphor. The phosphor absorbs the 254 nm radiation and then readmits the radiation by fluorescence producing a diffuse, even light at somewhat lower energy than the original arc. By varying the phosphor composition, a wide spectral range can be provided. The spectral distribution of two fluorescent light sources as well as a low-pressure mercury lamp, sunlight, and the UV absorption curve of ordinary borosilicate (Pyrex) glass is depicted in Figure V-12.

As noted previously, the medium that the light passes through before striking the test chemical is also important to the intensity and wavelength of the light reaching the test chemical. Table V-11 lists a variety of construction material used to make photochemical apparatus. The transmissions indicated should be taken into account when reviewing the studies listed in Table V-10.

While the use of artificial lamps is perfectly reasonable if the products of photolysis are of prime concern, difficulties exist when attempts are made to relate rates obtained with

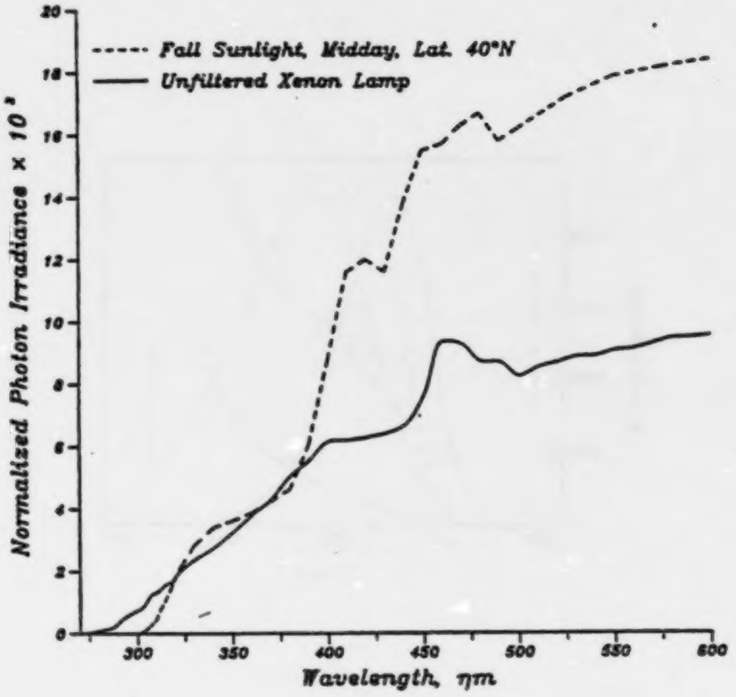


Figure V-11. Spectral irradiance of xenon lamp compared to fall sunlight (Zepp and Cline, 1977)

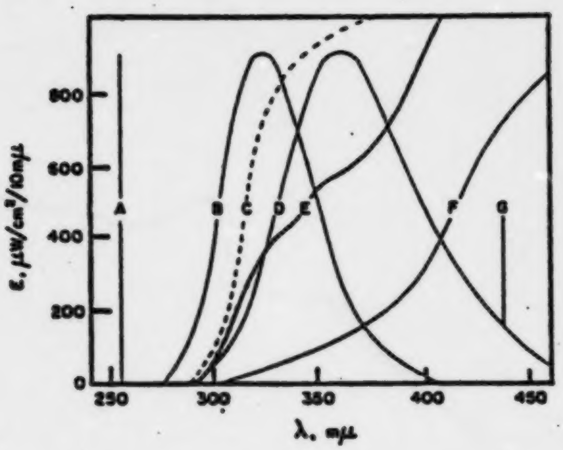


Figure V-12. Spectral distribution of a low-pressure mercury lamp (A,G); fluorescent sunlamp (B); fluorescent black-light (D); normal sunlight (E); daylight fluorescent lamp (F); and transmission spectra of borosilicate glass (C) (Crosby, 1969b).

Table V-11. Approximate Wavelength Limits for Transmission of Various Materials and Water at Room Temperature (Calvert and Pitts, 1966)

Material	Thickness (mm)	Approximate γ (nm) for Percent Transmission Indicated		
		50%	30%	10%
Window Glass (standard)	1	316	312	307
	3	330	323	314
	10	352	342	330
Optical (white crown) Glass	1.8	327	320	309
Pyrex (Corning 774)	1	306	297	280
	2	317	309	297
	4	330	319	310
Corex D	1	278	267	250
	2	288	280	267
	4	304	292	281
Corex A	2.9	248	243	240
Vycor 790	2			>254
Vycor 791	1	215	213	212
	2	223	217	213
	4	236	225	217
Quartz, crystal	5	185		
	10	193	192	186
Plexiglas (polymethylmethacrylate)	2.5	322	310	297
	5.0	338	325	311
	10.0	350	342	326
Water (distilled)	20	188	186	185
	40	192	188	186
	80	202	194	188

artificial light sources to the environment. This occurs even though the wavelength of light emitted by artificial light sources may correspond to sunlight since the intensity at each wavelength may differ considerably. Zepp (1981) has illustrated this point by computing the degradation rate of naphthalene, anthracene, and naphthacene in sunlight and in artificial light, and his results are presented in Table V-12. The three compounds absorb in different spectral regions, and it is clear that the results obtained in sunlight cannot be reproduced by any one lamp either on an absolute or a relative basis.

The use of sunlight for photodegradation studies has been advocated by Zepp and Cline (1977) who have described the effect of variables such as time-of-day, latitude, season, etc., and some of their results are illustrated in Figures V-13 and V-14. Figure V-13 reveals that latitudinal variation is small during the summer and appreciable during spring and fall. Furthermore, intensity variations increase with increasing distance from the tropical zone. Figure V-14 shows that the intensity of sunlight is fairly constant in the 4-hour period centered at noon, and decreases as expected in either direction. Zepp and Cline (1977) have prepared a computer program which calculates sunlight intensities as a function of season, latitude, longitude, time-of-day, depth of solution, and the thickness of the ozone layer. Thus, it is possible to relate photochemical rate data obtained with a given set of conditions to other environmental situations.

There are, however, certain problems associated with the use of sunlight as the source of irradiation. First, the variability

Table V-12. Relative Rate Constants Computed for Direct Photolysis of Naphthalene, Anthracene, and Naphthacene with Various Light Sources with Spectral Irradiance Normalized so that Integrated Irradiance (300-380 nm) is the Same for All Sources[a,b].

Light Source	Naphthalene	Anthracene	Naphthacene
Summer sunlight[c]	1	1	1
Winter sunlight[c]	0.55	1.02	1.25
Sunlamp	11.6	0.55	0.12
Blacklight	0.17	1.13	0.043
Medium-pressure mercury lamp	12.7	0.57	0.31
Xenon lamp	2.6	0.94	0.65

[a] The artificial light is assumed to be filtered so that wavelengths less than 295 nm are excluded.

[b] For each compound, relative rate constants were computed by ratioing to the rate constant for direct photolysis at midday during summer, latitude 40°N.

[c] Midday, latitude 40°C.

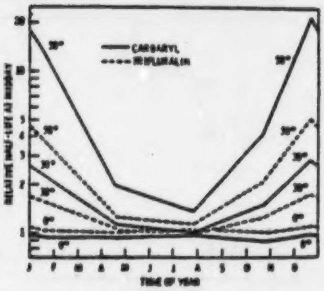


Figure V-13. Relative midday half-lives for direct photolysis of carbaryl and trifluralin at mid-season for several northern latitudes. (Zepp and Cline, 1977)

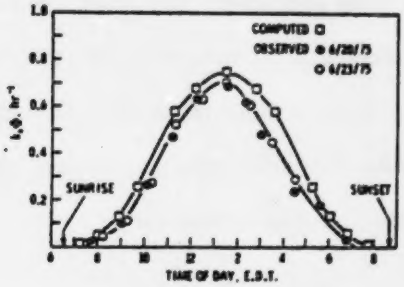


Figure V-14. Time-of-day dependence of direct photolysis of DMDE at Athens, GA. (Zepp and Cline, 1977)

of smog and cloud may lead to error, although this is generally less than a factor of 2 (Zepp, 1981). A more severe problem arises with chemicals which photodegrade slowly and require long exposure periods. In this situation, the intensity of the sunlight will change considerably during the measurement, and the observed rate will vary with the intensity. A procedure which circumvents both problems is to expose a reference compound at the same time and under the same conditions as the test compound. If the absorption spectrum of the reference compound and its degradation rate approximate those of the test compound, it is likely that reproducible photolysis rates will be obtained.

(ii) Solution Photochemistry

(a) Photochemical Equipment

A frequently used technique for studying the photochemistry of a chemical is to dissolve the material in a solvent before irradiation. Solvents such as water, methanol, ethanol, hexane, cyclohexane, and benzene are often used.

The photochemical equipment used by many pesticide photochemists includes (1) Crosby's sunlight-simulating laboratory photoreactor (Crosby and Tang, 1969a), (2) the quartz immersion well apparatus used by Plimmer _et_ _al_. (1967, 1970) and Rosen and Siewierski (1971, 1972), and (3) the Rayonet Photochemical Reactor used by Zabik and coworkers (Pape and Zabik, 1972a,b; Pape _et_ _al_., 1970). Crosby's apparatus is depicted in Figure V-15. The reactor is constructed of borosilicate glass and, therefore, the

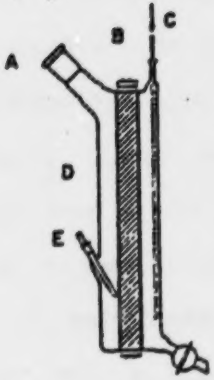


Figure V-15. Sunlight-simulating, laboratory photoreactor. Condenser joint (A), lamp (B), gas inlet (C), reaction chamber (D), and thermocouple well (E) (Crosby and Li, 1969).

wavelength of light reaching the solution is no longer than 290 nm (see Curve C, Figure V-12). A germicidal (low-pressure mercury) lamp may be used, but a fluorescent lamp is preferred (Crosby and Li, 1969).

Crosby and Tang (1969b) have also used a similar device equipped for continuous extraction, in their study of 1-naphthaleneacetic acid (Figure V-16). This allowed the trapping of the photosensitive intermediate photoproducts before they were degraded.

The quartz immersion well apparatus depicted in Figure V-17 is more flexible in terms of the light energy that can be imparted to the solution. Normally a medium or high pressure lamp is placed in the immersion well. The quartz well will transmit light >180 nm but often the light is attenuated with various filter sleeves (Vycor, >210 nm; Corex, >250 nm; and Pyrex, >280 nm).

The Rayonet apparatus differs from the immersion well apparatus in that the light is provided by a bank of low pressure mercury or fluorescent lamps which surround the reactor (λ max. of the available lamps is 254, 300, and 350 nm). Alternatively, the solution is placed in a number of cylindrical tubes, which are rotated (Merry-Go-Round Reactors); construction materials may vary from quartz to Pyrex glass.

Much simpler containers such as petri dishes have also been used for solution photochemistry. For example, Crosby and Tang (1969a) used a borosilicate glass baking dish

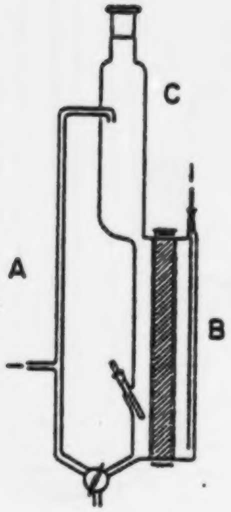


Figure V-16. Photoreactor (B) equipped with a gas lift (A) for continuous extraction (C) (Crosby and Tang, 1969b).

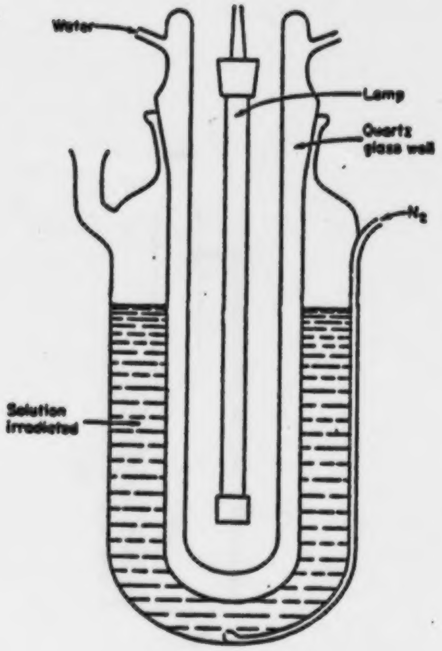

Water
Lamp
Quartz glass well
N_2
Solution irradiated

Figure V-17. Quartz immersion well photochemical reactor (Kearney et al., 1969).

covered with a perforated clear plastic wrap (transmits light >200 nm), when irradiating an aqueous solution with sunlight. Aly and El-Dib (1971) used open petri dishes when they irradiated a number of carbamate insecticides in aqueous solution with germicidal lamps (254 nm). Henderson and Crosby (1968) used an inverted, round-bottom, quartz flask for sunlight irradiation of an aqueous solution of dieldrin.

In the event that sunlight is used, the container used to expose the solution may affect the photolysis rate. Vessels such as test tubes, cells, or flasks increase the light path length through internal reflection by a factor of up to 2 (Zepp, 1981). Shallow, flat containers are therefore preferable and more nearly approximate actual environmental exposures. However, if a reference compound is used along with the test chemical, the shape and size of the container makes little difference to the result.

(b) Methods Used for Measuring Photolysis Rates

The procedures used for photochemical rate measurements depend primarily upon the sensitivity of the analytical technique. While photolysis of aqueous solutions is the preferred procedure, the limited solubility of some substrates preclude the use of aqueous solutions. In these cases, organic solvents, mixtures of organic solvents and water, and suspensions of the test chemical in water have been utilized. Thus cyclohexane, methanol (Chittim _et_ _al_., 1978), hexane, aqueous acetonitrile (Zepp, 1976), isooctane (Bunce et al., 1978), among other solvents, have been used. Of these, a dilute solution (≈1% v/v) of acetonitrile in water is preferred since the dielectric constant of acetonitrile is close to that of water, and acetonitrile does not usually participate in radical transfer reactions.

There are two methods that have commonly been utilized for rate measurements. First, solutions of the substrate have been photolyzed either with artificial light sources or with sunlight, and the rate has been measured. In dilute solutions, the kinetics are of the first-order if the intensity of irradiation is constant (Calvert and Pitts, 1966; Zepp, 1978). The second method involves the measurement of quantum yield Φ_λ which is defined as follows:

$$\Phi_\lambda = \frac{\text{no. of molecules that photoreact}}{\text{no. of photons absorbed}}$$

A monochromatic light source emitting light of an environmentally relevant wavelength such as 300 or 310 nm is chosen and the extent of degradation per unit time is measured in a merry-go-round apparatus. Alternatively, a medium pressure mercury lamp is used with appropriate filters to isolate the wavelengths of interest. An optical bench may also be used in place of the merry-go-round apparatus, although this practice is more prevalent in basic photochemical research than in environmental photochemistry. An actinometer (a compound which photoreacts with a known quantum yield) is irradiated at the same time as the sample and the light intensity is calculated from the extent of reaction of the actinometer (Calvert and Pitts, 1966; Zepp, 1978). The quantum yield can be readily calculated from these data. It is now assumed that the quantum yield does not vary with the wavelength (i.e., light of different wavelengths initiate the same photoprocess). In general, this is not a severe assumption, and in one example, Banerjee et al. (1978) have found that disappearance quantum yields for benzidine and 3-chlorobenzidine are invariant between 254 nm and 300 nm. The degradation rate of the test compound in sunlight may be calculated from the quantum yield, the absorption spectrum of the compound at >290 nm and the intensity of sunlight at the point of interest. Zepp and Cline (1977) have compared rates calculated from the quantum yield to those measured in sunlight for ten pesticides and pesticide derivatives and have found the two sets of values to be within ± 30% of each other.

(c) Products of Photolysis

The majority of environmental photochemical studies to date have been concerned with agricultural chemicals. These compounds frequently contain a chloroaromatic moiety, and consequently, a large body of data on the photolysis of chloroaromatics is available. In general, carbon-chlorine bond cleavage occurs either homolytically to yield reduced products or heterolytically to give substituted materials. The former process is favored in organic media, where the initially formed aryl radical may abstract hydrogen from the organic solvent as shown below.

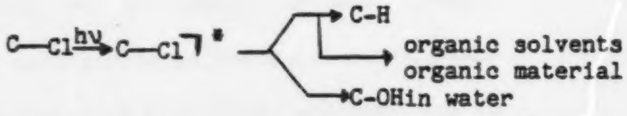
$$\text{C-Cl} \xrightarrow{h\nu} \text{C}\cdot \xrightarrow{\text{R-H}} \text{C-H} + \text{R}\cdot$$

Thus, photolysis of chlorinated p-terphenyls in methanol (Chittim et al., 1978), chlorobiphenyl in aqueous acetonitrile (Bunce et al., 1978), methoxychlor in hexane (Zepp et al., 1976), all lead to reduced products. There are, however, exceptions to the above mechanism. Dechlorination of chlorobiphenyls in ether containing 2% trifluoroacetic acid (Nordblom and Miller, 1974) and chlorobenzidine in water (Banerjee et al., 1978) does not proceed via bond homolysis, and there is evidence that degradation of chlorobiphenyls in isooctane may involve electron transfer processes (Bunce, 1978).

Photolysis of chloroaromatics in water or other solvents capable of nucleophilic attack usually leads to substituted products. Havinga and Cornelisse (1976) have extensively studied this mechanism and have provided evidence that the process involves simple nucleophilic displacement of chloride from the excited state by suitable anions. Examples of this reaction abound, and are presented in Table V-10.

Two recent studies have explored the effect of organic materials on the photolysis of chloroaromatics in water. For example, the sodium salt of 2,4-D suffers hydroxylation during photolysis in water (Crosby and Tutass, 1966), whereas photolysis of commercial-formulated emulsion concentrates gives rise to reduced products (Que Hee _et_ _al_., 1979). Similarly, Tanaka et al. (1979) has shown that addition of nonionic surfactants to solutions of 3-(4-chlorophenyl)-1,1-dimethyl urea in water promotes reductive dechlorination at the expense of hydroxylation. It is postulated (Que Hee _et_ _al_., 1979; Tanaka _et_ _al_., 1979) that the photoprocesses occur in micelles formed in aqueous solution. A similar effect may occur when compounds which tend to sorb to sediment are photolyzed in the presence of suspended sediment. Miller and Zepp studied the photolysis of DDE (the dehydrochlorinated derivative of DDT) in the presence of sediment and concluded that the sorbed compound was in an environment that was a better hydrogen donor than water. These studies are of relevance since environmental waters may contain dissolved or suspended organic matter, and photoproduct distribution in natural water may be appreciably different from that expected from laboratory studies which utilize pure water.

In summary, the photochemistry of chloroaromatic compounds may be represented by the following scheme.



In pure water, the likely process is hydroxylation, whereas in organic solvents or media containing organic matter, reductive dehalogenation is preferred. The photochemistry of environmentally important compounds which do not incorporate the chloroaromatic unit is diverse, and is not easily amenable to classification. Some representation examples are shown in Table V-10.

(d) Photosensitized Processes

Photosensitized processes occur when a compound (sensitizer) absorbs light and transfers energy to another compound which then undergoes reaction (Neckers, 1967). The most important environmental photosensitization reaction is photooxidation, with naturally occurring materials in water acting as the photosensitizers. There are two major classes of sensitized reactions. First, the sensitizer interacts with another compound, which then reacts further. Second, the sensitizer reacts with oxygen to yield singlet oxygen, which then reacts with other molecules (Foote, 1978; Davidson, 1979).

Interaction of sensitizer with compound may occur in several different ways. First, simple energy transfer (Neckers, 1967) may occur, provided that the triplet state energy of the compound does not exceed that of the sensitizer. However, instances are known (Zepp _et_ _al_., 1976) where photosensitization occurs even when the triplet energy of the compound is too high to allow energy transfer from the sensitizer, and in these cases reaction between the sensitizer and compound may occur (Zepp and Baughman, 1978).

The second process (i.e., transfer of energy of sensitizer to oxygen) is of greater environmental relevance since the excited state energy of oxygen is low (Zepp and Baughman, 1978), the concentration of oxygen in environmental waters is high, and energy transfer to oxygen from the sensitizer is therefore facile. The process usually leads to singlet oxygen which then reacts directly with the pollutant or leads to other reactive species, which attack the pollutant. The formation of singlet oxygen upon irradiation of several natural waters has been observed (Zepp _et_ _al_., 1977), and it is likely that natural photosensitizers may be derived from humic materials present in water (Zepp _et_ _al_., 1976).

The products from sensitized processes usually differ from those obtained from direct photolysis. Thus, direct photolysis of bromacil (5-bromo-3-sec-butyl-6-methyluracil) yielded 5-bromo-6-methyluracil (Moilanen and Crosby, 1974), whereas photolysis in the presence of methylene blue gave hydantoin derivatives (Acher and Dunkelbum, 1979). Similarly, 3,4-dichloroaniline gave tetrachloroazobenzene upon sensitized photolysis, whereas none of this material resulted during direct irradiation (Miller _et_ _al_., 1980).

(e) Other Factors Influencing Photodegradation

In the previous sections, the effect of light intensity and wavelength, solvent composition, and photosensitizers on photochemical rates and products have been discussed. Other factors that may influence photoprocesses are pH, the presence of suspended sediments, and other materials that may interact with the compound. For example, suspended materials attenuate the intensity of the incident light. Miller and Zepp (1979) and Oliver et al. (1979) have shown that sediments shield the pollutant from available light. However, in certain cases (Miller and Zepp, 1979), light scattering by sediment may increase the path length of the light and thereby lead to enhanced photolysis.

The pH of the reaction solution can have a considerable effect on the photochemical or chemical process that takes place. For example, Langford et al. (1973) found that the quantum yield decreased linearly with increasing pH over the range 2-12 in the photodecomposition of copper complexes of NTA irradiated with 350 nm light (Rayonet unit). Crosby and Leitis (1973) reported that the principal product from photolysis of trifluralin was completely different under acidic compared to basic conditions (see Figure V-18). Crosby and Wong (1973) also noted that the rate of 2,4,5-T photolysis was somewhat more rapid at pH 8 than at pH 3. More recently, Banerjee et al. (1978) have shown that the disappearance quantum yield of 3,3'-dichlorobenzidine decreases with increasing pH.

(f) Protocols for Photodegradation

Unlike the protocols developed for hydrolysis, the corresponding protocols for photolysis developed by various

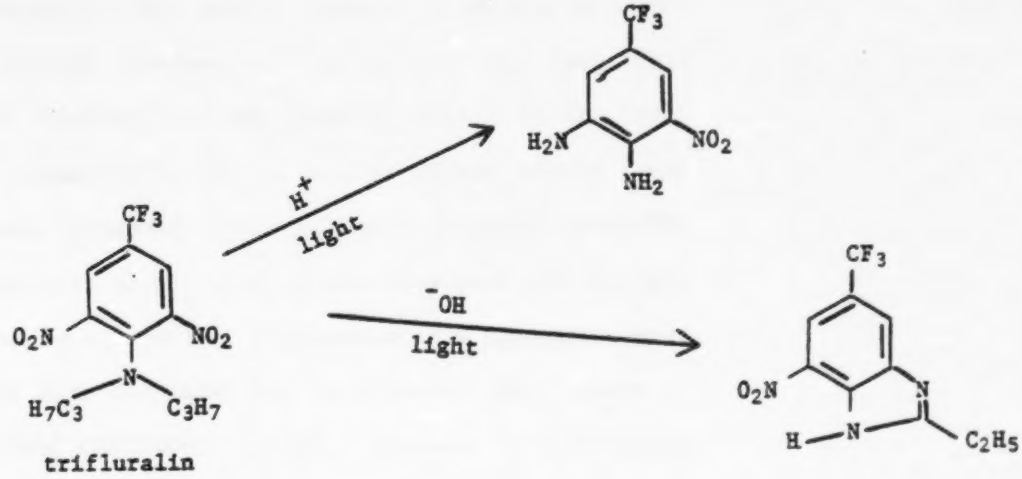

CF3
H2N
NO2
NH2
H+
light
CF3
O2N
NO2
N
H7C3
C3H7
trifluralin
-OH
light
CF3
O2N
N
H
N
C2H5

Figure V-18. Basic and acidic photolysis of trifluralin (Crosby and Leitis, 1973).

agencies and organizations differ considerably from one another (Sosebee, 1980). The main point of difference is the source of irradiation, and as such, the results obtained from one protocol may not be comparable to those obtained from others. The EPA-FIFRA (Federal Register, 1978) and the ASTM (ASTM, 1981) protocols recommend the use of either sunlight or artificial light. Since the correspondence of artificial light to sunlight is compound dependent (Zepp, 1981), it is highly unlikely that studies with artificial light can be easily related to the environment. The EPA (PMN-TSCA) (Federal Register, 1979) protocols specify sunlight as the source of irradiation. It is anticipated that these protocols will enventually incorporate an actinometer to measure the intensity of sunlight, but this feature is unavailable at present. The EPA (PMN-TSCA) protocols are probably the most detailed of the available protocols, and are described below and summarized in Figure V-19.

The protocol requires the use of compounds that are >99% pure, sterilized water, and sterilized glassware. Solutions are prepared such that (i) the compound is present at less than half of its solubility in water and less than 1×10^{-5} M, and (ii) the absorbance of the solution in a 1 cm cell is <0.02 at wavelengths exceeding 290 nm. For relatively water insoluble compounds, a solution prepared in acetonitrile is added to water such that the acetonitrile content does not exceed 1%. For compounds capable of undergoing acid-base equilibria, the experiments are carried out

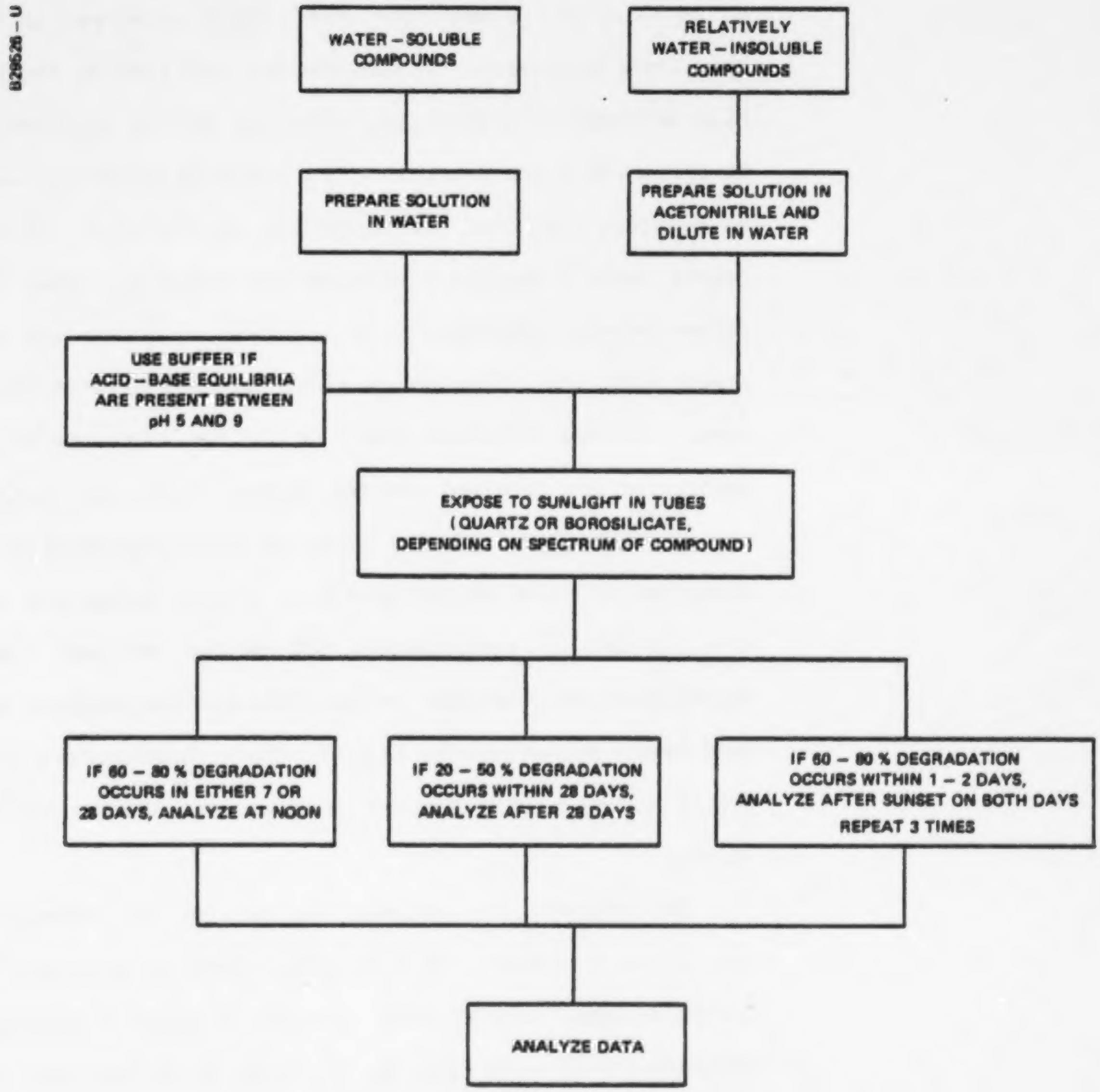

829526-U
WATER – SOLUBLE COMPOUNDS
RELATIVELY WATER – INSOLUBLE COMPOUNDS
PREPARE SOLUTION IN WATER
PREPARE SOLUTION IN ACETONITRILE AND DILUTE IN WATER
USE BUFFER IF ACID – BASE EQUILIBRIA ARE PRESENT BETWEEN pH 5 AND 9
EXPOSE TO SUNLIGHT IN TUBES (QUARTZ OR BOROSILICATE, DEPENDING ON SPECTRUM OF COMPOUND)
IF 60 – 80 % DEGRADATION OCCURS IN EITHER 7 OR 28 DAYS, ANALYZE AT NOON
IF 20 – 50 % DEGRADATION OCCURS WITHIN 28 DAYS, ANALYZE AFTER 28 DAYS
IF 60 – 80 % DEGRADATION OCCURS WITHIN 1 – 2 DAYS, ANALYZE AFTER SUNSET ON BOTH DAYS
REPEAT 3 TIMES
ANALYZE DATA

Figure V-19. PMN-TSCA Test Protocols for Aqueous Photodegradation

at pH 5, 7, and 9 with the same buffers described in the hydrolysis protocols. If the compound exhibits an absorption spectrum at wavelengths exceeding 340 nm, cylindrical borosilicate glass tubes of 11 mm inside diameter are used as reaction vessels. If absorption occurs below 340 nm, quartz tubes of similar dimensions are required. Tubes are filled almost completely with the test solutions and are sealed with greaseless ground glass stoppers or with screw caps. Control solutions identical to the solutions to be photolyzed are prepared and the control tubes are wrapped with aluminum foil. All the tubes are placed outdoors in an area free of shade and reflection on a black background and are inclined at approximately 30° to the vertical. All experiments are conducted in duplicate and the analyses are made with a method precise to ± 5%. The experiments are conducted during a warm time of year and are started before sunrise.

The schedule for analysis varies with the photolysis rate of the compound. If photolysis occurs to an extent of 60-80% between 7 and 28 days, the test solution is analyzed periodically at noon. A point is chosen where less than 40% of the initial compound concentration remains, and the photolysis rate constant is calculated from the equation

$$\ln \frac{C_o}{C} = k_p t$$

where Co and C reflect compound concentration at t=o and t=t, respectively, and k_p is the photolysis rate constant.

The photolysis half-life ($t_{1/2}$) is available from the equation

$$t_{1/2} = \frac{\ln 2}{k_p}$$

For compounds that undergo 20-50% photodegradation in 28 days, the analyses are performed at 28 days, and the rate constant and half-life is calculated as above. Compounds that degrade to an extent of 60-80% within one or two days are analyzed after sunset on the first and second days. The experiments are carried out three times (duplicate experiments at each time), and k_p values are obtained from the initial concentration and the concentration remaining after a full day.

2. Hydrolytic Alterations

Hydrolysis represents a major pathway for the degradation of xenobiotics in the aquatic environment. Unlike photodegradation, which requires special techniques for environmental studies, the methods used in hydrolysis studies are virtually identical to those routinely followed in physical-organic chemistry. Consequently, hydrolytic measurements are fairly easily made, and the data can be interpreted and related to environmental situations with a high degree of reliability.

Experimental measurements usually involve maintaining the compound of interest in an aqueous buffered solution of constant ionic strength and temperature, and following the disappearance of substrate with time. Dilute solutions of the compound are used, and the observed kinetics are usually of the first-order. The effect of pH, temperature, and occasionally ionic strength on the observed rate constant is measured (Mill, 1980), and these data are sufficient to allow environmental assessment of the hydrolytic stability of the chemical to be made.

The observed hydrolysis rate constant (k_{obs}) is given by the following expression where k_a, k_b, and k_n reflect acid, base, and uncatalyzed processes.

$$k_{obs} = k_a [H_3O^+] + k_n + k_b [OH^-]$$

The rate-pH profile varies with the magnitude of the various rate constants in the above equation, and a typical rate profile is shown in Figure V-20. It is important to note that the valley of the rate profile does not necessarily occur at pH=7, but where $k_a [H_3O^+] = k_b [OH^-]$, and therefore depends upon the relative magnitude of k_a and k_b (Bell, 1959).

The effect of temperature on rate can be determined by measuring the observed rates at various temperatures. The following equation

$$k_{obs} = Ae^{-\Delta G/RT}$$

where A and G are the frequency factor and activation energy, respectively, applies, and a plot of ln (k_{obs}) vs. 1/T gives a straight line of slope ($-\Delta G/R$). Values for ΔG typically range from 15-25 kcals/mole (Freed et al., 1979; Wolfe et al., 1976), which implies that the rate increases by a factor of 1.5-2 for every 5° temperature rise at typical environmental temperatures.

Table V-13 provides representative examples of a number of recent environmental studies. In some of these studies (Wolfe et al., 1976, 1977), rates measured in both buffered solutions and natural waters were found to agree closely. In general, base catalysis appears to be more important than acid

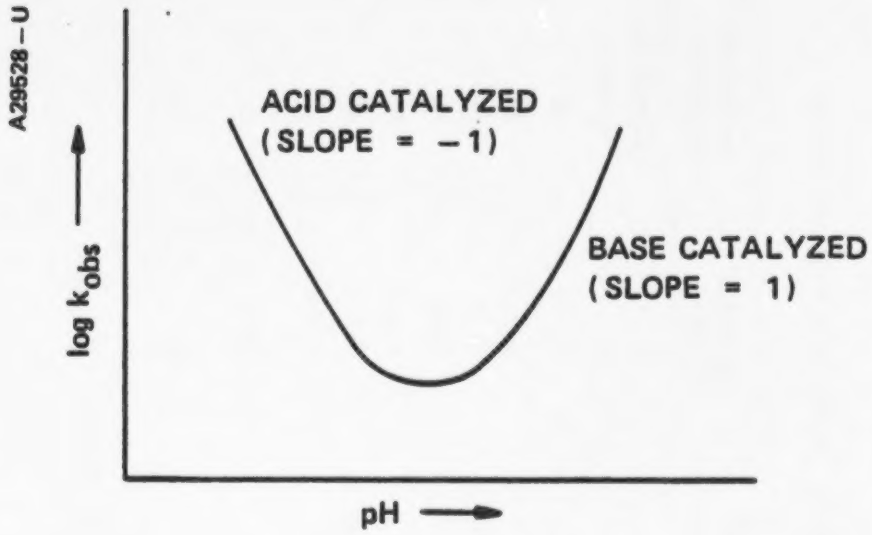


Figure V-20. Hydrolysis Rate Constant-pH Profile

Table V-13. Hydrolysis Kinetic Studies

Chemical Studied	Nature of Catalyst[a]	pH Range Studied	Analytical Procedure	Reference
Phenylamide pesticides	base	>5	Colorimetry	El-Dib and Aly, 1976
Thiofanox	base	10	^{14}C-TLC	Chin et al., 1976
Organophosphate pesticides	base	6.1-7.4	GC	Freed et al., 1979
Captan	base	2-8.3	GC	Wolfe et al., 1976
Methoxychlor	base	3-13	GC	Wolfe et al., 1977
DDT	base	4-12	GC	Wolfe et al., 1977
Tetrachloroinphos	base	5.8-9	GC	Akhtar, 1977
2,4-D Esters	acid, base	1-8	GC	Zepp et al., 1975
Propyzamide	base	4.8-8.3	GC	Bastide and Coste, 1978
Fenitrothion	base	3-11	GC	Greenhalgh et al., 1980
RDX	base	>12	GC	Hoffsommer et al., 1977

[a] Not necessarily effective at environmental pH levels.

catalysis (Mill et al., 1980) in environmental studies, although this probably reflects the nature of the compounds studied to date.

Finally, hydrolysis rates may be used to derive structure-reactivity correlations. The procedures used are generally of the Hammett-Taft variety (Mabey and Mill, 1978), which have been successfully used for several years (Hammett, 1970), and can be easily and reliably applied.

a. Protocol for Hydrolysis Studies

Several agencies and organizations such as the EPA, ASTM, and OECD have developed or are developing protocols for hydrolysis studies. The techniques used in the protocols are the same or are very similar to one another, and the differences between them are primarily procedural in nature (Sosebee, 1980). For example, the OECD and the ASTM protocols are tiered, with the complexity of the tests increasing with the level of the tier. One of the more comprehensive protocols has been described by the EPA (Premanufacture Testing - Toxic Substances Control) (Federal Register, 1979), and the methods used are summarized below and in Figure V-21.

The protocol requires the use of compounds that are >99% pure, pure sterile water, sterilized glassware, and a constant temperature bath at 25°C and precise to ± 1°C. Three buffers of pH 5 (acetate), 7 (phosphate), and 9 (borate) are prepared such that the buffer concentration is 0.01 M, and the pH is verified at 25°. For water soluble compounds, an aqueous solution of the test compound is diluted with buffer such that the final concentration of the compound is less than half its water solubility and also less than 1×10^{-3} M. For relatively insoluble compounds, a solution is prepared in acetonitrile and then diluted with buffer such that the acetonitrile content is 1% or less, and the concentration of the compound does not exceed half its water solubility or 1×10^{-3} M. If the test compound exhibits an absorption spectrum in the visible

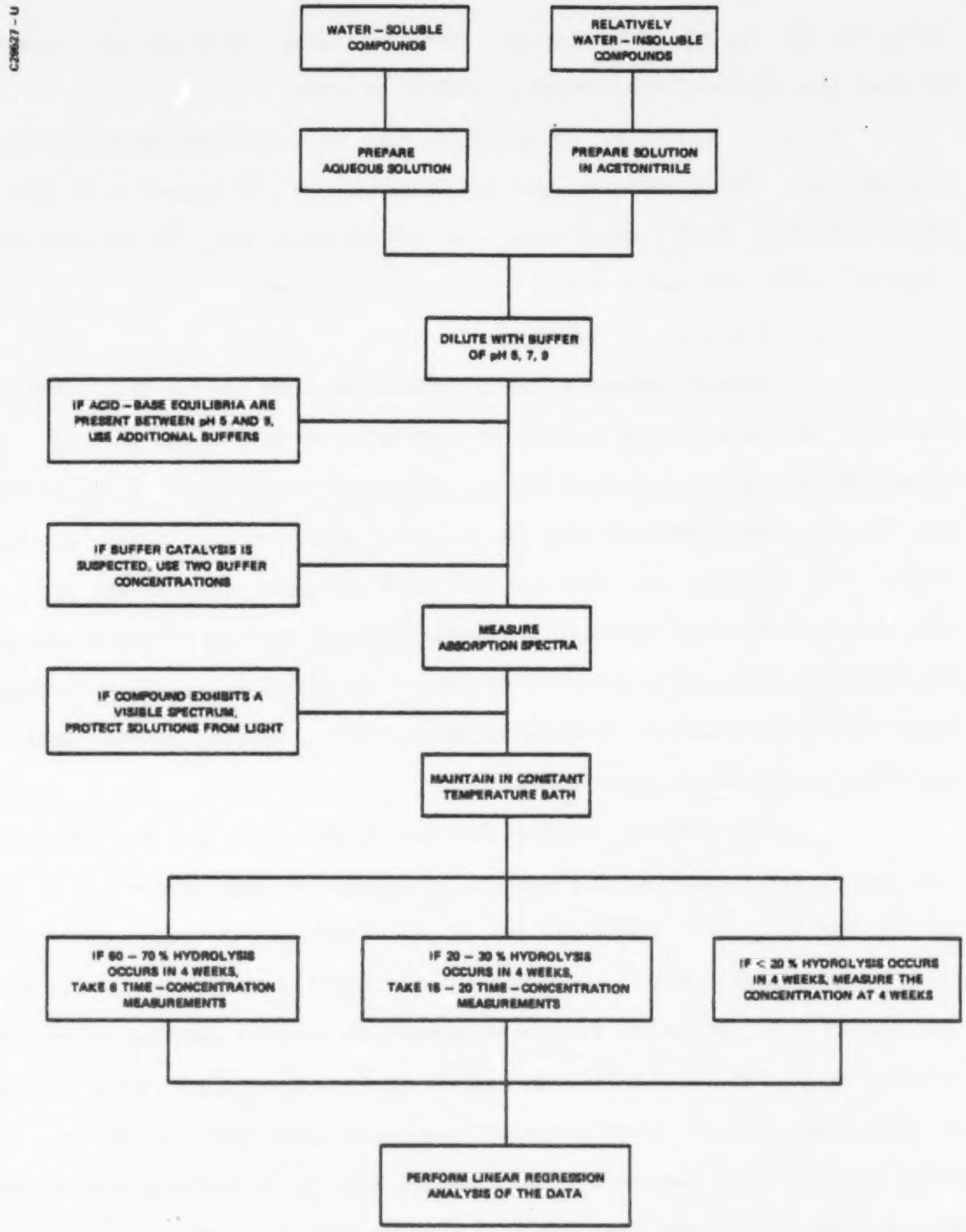

C29627-U
WATER – SOLUBLE COMPOUNDS
RELATIVELY WATER – INSOLUBLE COMPOUNDS
PREPARE AQUEOUS SOLUTION
PREPARE SOLUTION IN ACETONITRILE
DILUTE WITH BUFFER OF pH 5, 7, 9
IF ACID – BASE EQUILIBRIA ARE PRESENT BETWEEN pH 5 AND 9, USE ADDITIONAL BUFFERS
IF BUFFER CATALYSIS IS SUSPECTED, USE TWO BUFFER CONCENTRATIONS
MEASURE ABSORPTION SPECTRA
IF COMPOUND EXHIBITS A VISIBLE SPECTRUM, PROTECT SOLUTIONS FROM LIGHT
MAINTAIN IN CONSTANT TEMPERATURE BATH
IF 60 – 70 % HYDROLYSIS OCCURS IN 4 WEEKS, TAKE 6 TIME – CONCENTRATION MEASUREMENTS
IF 20 – 30 % HYDROLYSIS OCCURS IN 4 WEEKS, TAKE 15 – 20 TIME – CONCENTRATION MEASUREMENTS
IF < 20 % HYDROLYSIS OCCURS IN 4 WEEKS, MEASURE THE CONCENTRATION AT 4 WEEKS
PERFORM LINEAR REGRESSION ANALYSIS OF THE DATA

Figure V-21. Schematic of Protocol for Hydrolysis Studies

region, all experiments are conducted with opaque glassware or in the presence of red or amber light.

The hydrolysis experiments are carried out in ungreased stoppered volumetric flasks which are filled almost completely, and sealed if volatile compounds are used. The flasks are submerged in the constant temperature bath and the solutions are analyzed periodically with an analytical method that provides a precision of at least ± 5%.

If hydrolysis occurs to an extent of 60-70% or more in four weeks, a minimum of six time-concentration points are taken between 20 and 70% reaction. For slower reactions that proceed to an extent of 20-30% conversion in four weeks, 15-20 time-concentration points are taken. If less than 20% hydrolysis occurs in four weeks, the solution is analyzed at that time.

All experiments are conducted in duplicate. Further experiments are conducted in two cases: (i) when the compound participates in acid-base equilibria in the pH 5-9 region, and (ii) when general acid-base catalysis is suspected. In the former case, additional points between pH 5-9 are taken to better define the pH-rate profile. In the latter case, measurements are made with two buffer concentrations at the same pH. If identical rates are obtained, the possibility of buffer catalysis is eliminated.

The data are fitted to the following equation

$$\ln C_t = \ln C_o - kt$$

where C_t and C_o represent substrate concentration at time t=t and t=o, respectively, and k is the first-order rate constant. The rate constant is derived through linear regression analysis, and the half-life ($t_{1/2}$) is calculated from the following equation

$$t_{1/2} = 0.693/k$$

3. Oxidative Alterations

Although oxidation is almost certainly important in the environment, little is known about oxidative processes, and methods to measure the rates of oxidation have not been standardized. There are two difficulties associated with chemical oxidation studies. First, it is difficult to separate chemical oxidation from photo-induced oxidation, and second, a variety of oxidizing agents may occur in the environment, and these have not been characterized or quantified to a sufficient extent.

In an attempt to quantify environmental oxidative processes, Mill et al. (1979, 1980) studied the oxidation of cumene and pyridine by the hydroxyl and the alkylperoxy radical. Hydroxyl radicals were generated from the photolysis of hydrogen peroxide in water and the alkylperoxy radicals were obtained from the decomposition of azo-bis(2-carbomethoxy propane). Their results showed that while hydroxyl radicals are highly reactive, their concentration is too low to be of environmental significance. On the other hand, the less reactive alkylperoxy radical is more abundant in natural waters, and it is possible that compounds such as phenols, anilines, hydroxylamines, and hydroxyquinones may be oxidized in the environment by this species. It must be noted, however, that the generality of the techniques used in this study remains to be established, and it is likely that the development of general protocols will require several additional studies to be performed.

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